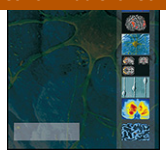
Neuroscience Letters 495 (2011) 173–177

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet



Further antinociceptive effects of myricitrin in chemical models of overt nociception in mice

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ARTICLE INFO

Article history:
Received 8 October 2010
Received in revised form 19 January 2011
Accepted 3 February 2011

Keywords:
Flavonoid
Myricitrin
Nociception
Bradykinin
Transient receptor potential
Mice

ABSTRACT

The present work explored the antinociceptive effects of the flavonoid myricitrin in models of overt nociception triggered by intraplantar injection of chemical algogens into the hind paw of mice. The nociception induced by bradykinin (3 nmol/paw i.pl.) was abolished by prior treatment with myricitrin (10–100 mg/kg, i.p.) with ID_{50} of 12.4 (8.5–18.1) mg/kg. In sharp contrast, myricitrin failed to affect the nociception elicited by prostaglandin E_2 (3 nmol/paw i.pl.). Cinnamaldehyde (10 nmol/paw i.pl.)-induced nociception was reduced by myricitrin (100 mg/kg, i.p.) and camphor (7.6 mg/kg, s.c.) in 43 ± 10% and 57 ± 8%, respectively. Myricitrin (30–100 mg/kg, i.p.) and amiloride (100 mg/kg, i.p.) inhibited nociceptive responses induced by acidified saline (pH 5/paw i.pl.), with ID_{50} of 22.0 (16.1–30.0) mg/kg and inhibition of 71 ± 6% and 64 ± 5%, respectively. Moreover, myricitrin (10–30 mg/kg, i.p.) and ruthenium red (3 mg/kg, i.p.) significantly reduced the nociception induced by menthol (1.2 μmol/paw i.pl.) with the mean ID_{50} of 2.4 (1.5–3.7) mg/kg and inhibition of 95 ± 3% and 51 ± 7%, respectively. In addition, myricitrin administration (30 and 100 mg/kg, i.p.) markedly reduced menthol-induced mechanical allodynia. However, myricitrin (100 mg/kg, i.p.) prevented (only in time of 60 min) cold allodynia induced by menthol. Collectively, the present results extend prior data and show that myricitrin promotes potent antinociception, an action that is likely mediated by an inhibition of the activation of nociceptors by bradykinin and TRPs agonist (i.e. cinnamaldehyde, acidified saline and menthol), probably via inhibition of PKC pathways. Thus, myricitrin could constitute an attractive molecule of interest for the development of new analgesic drugs.

Acute nociceptive pain is the consequence of primary afferent nociceptive C and Aδ fibers activation by intense mechanical, chemical, or thermal stimuli [18]. Thus, such noxious stimuli excite peripheral nociceptors by activation of distinct types of ionotropic channels and metabotropic receptors [18]. In fact, transient receptor potential (TRP) and acid-sensing ion channels (ASIC) play important roles in generating nociceptive signals in response to various specific noxious stimuli [10]. In addition, following tissue injury or during certain inflammatory processes occurs the release of pro-inflammatory mediators, namely bradykinin, prostaglandins and protons that contribute to elicit nociceptive signals as well as to promote hyperalgesia and allodynia [7,18]. Finally, the activity of some of these receptors can be facilitated (or upregulated) by protein kinases A and C (PKA and PKC), which sensitize them or amplify their responses by phosphorylating key residues in their structures, as well as of other proteins implicated in nociceptive signaling pathways in sensory neurons [32,33].

Although a considerable number of antinociceptive drugs are available, novel substances could contribute to our current understanding of the nociceptive signaling pathways to improve the treatment of painful conditions. Many plant-derived substances are attractive sources for developing new analgesic agents, among these, myricitrin, a naturally occurring flavonoid obtained from genus *Eugenia* displays marked anti-inflammatory and antinociceptive effects in rodents [24–27]. Our group has shown that myricitrin inhibited nociception caused by acetic acid and by intraplantar injection of glutamate, capsaicin and phorbol myristate acetate (PMA) in mice [25]. In addition, myricitrin reduced mechanical hyperalgesia induced by intraplantar injection of bradykinin in rats as well as the inflammatory and neuropathic allodynia caused by intraplantar injection of complete Freund's adjuvant and

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0304-3940
doi:10.1016/j.neulet.2011.02.007

by partial sciatic nerve ligation in mice [25,26]. Interestingly, the mechanisms involved in the antinociceptive action of myricitrin include inhibition of PKC and of p38 MAPK phosphorylation [25,27].

Here, we evaluated the effect of myricitrin on nociception triggered by intraplantar injection of bradykinin, prostaglandin E2, protons (activator of ASIC/TRPV1 channels), cinnamaldehyde (agonist of TRPA1 channel), menthol (agonist of TRPM8 channel). Finally, we evaluated the effect of myricitrin on cold and mechanical allodynia induced by intraplantar injection of menthol in mice.

Male Swiss mice (25–35 g) were housed (22 ± 2 °C, 12 h light–dark cycle) with food and water *ad libitum*. Mice were acclimatized to the laboratory for at least 1 h before testing. All experiments were previously approved by the UFSC's Committee on the Ethical Use of Animals, and conducted under the ethical guidelines of the International Association for the Study of Pain [42].

First, we investigated whether myricitrin would be able to antagonize bradykinin- and prostaglandin-induced nociception in the mouse paw. Animals used were individually adapted into a glass funnel. Myricitrin (1–100 mg/kg, i.p.) or vehicle (control, 10 ml/kg, i.p.) was administered 30 min before intraplantar (i.pl.) injection of the chemical algogens [12,19]. Following myricitrin or vehicle treatment, mice received a 20 μl i.pl. injection of either bradykinin (3 nmol/paw) or prostaglandin E2 (3 nmol/paw). Immediately after the injection, each animal was placed into individual glass cylinder of 20 cm and paw licking or biting was measured for 15 min, and considered as indicative of nociception.

In another set of experiments, to test if TRPA1, TRPM8 and ASIC receptors constitute potential specific targets for the antinociceptive actions of myricitrin, we tested the effects of this flavonoid against nociceptive responses elicited by activators of each channel. To this effect, following treatment with myricitrin, camphor [7.6 mg/kg, subcutaneous (s.c.) used as positive control], amiloride (100 mg/kg, i.p. used as positive control), ruthenium red (3 mg/kg, i.p. used as positive control) or vehicle, mice received a 20 μl intraplantar (i.pl.) injection of either cinnamaldehyde (10 nmol/paw), acidified saline (2% acetic acid in 0.9% saline, pH 5/paw) or menthol (1.2 μmol/paw). Paw licking or biting were recorded for 5 min (for cinnamaldehyde), 15 min (for acidified saline) or 20 min (for menthol).

Myricitrin (30 mg/kg) was administered by i.p. route at different times (15–240 min) before i.pl. injection of menthol to determine the duration of its antinociceptive effect. Next, we examined the influence of myricitrin (30 and 100 mg/kg, i.p.) in menthol-induced mechanical and cold allodynia. For assessment, mice were placed in clear plexiglas observation chambers (9 cm × 7 cm × 11 cm) on elevated wire mesh platforms. Animals were habituated to the chamber for 30 min. Hind paw responsiveness to mechanical or cold stimuli were assessed before menthol (1.2 μmol/paw) injection and then again, repeatedly, at several time points thereafter, but animals were exposed only to a single modality of sensory stimulus.

Mechanical allodynia was evaluated as the withdrawal response frequency to 10 applications of 0.4 g of von Frey hairs (Stoelting, Chicago, USA) [6]. Cold allodynia was assessed through spraying 50 μl of acetone on the plantar surface of the hind paw. Behavioral response was analyzed during 20 s and then recorded in scores: 0 – no response; 1 – quick withdrawal, flick or stamp of the paw; 2 – prolonged withdrawal or repeated flicking of the paw; 3 – repeated flicking of the paw with licking directed at the ventral side of the paw. Acetone application was repeated three times for each hind paw, in 5 min intervals, and the sum of three scores was used for data analysis [13].

The following substances were used: myricitrin was isolated from the plant of genus *Eugenia* in the Department of Chemistry, Federal University of Santa Catarina, Brazil. Analysis of the 1H NMR and 13C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure [1]. The chemical purity of myricitrin (more than 98%) was determined by GC/HPLC. Myricitrin was dissolved in Tween 80 plus saline. The final concentration of Tween did not exceed 10% and did not cause any effect per se. Bradykinin, prostaglandin E2, cinnamaldehyde, menthol amiloride, camphor and ruthenium red were from Sigma–Aldrich (St. Louis, MO). All other chemicals were of analytical grade and obtained from standard commercial suppliers. Drugs were dissolved in 0.9% NaCl solution, with the exception of menthol, which was dissolved in 1.6% ethanol/0.01% Tween 80 in saline. In both of these conditions, the final solutions containing ethanol failed to cause any nociceptive effects per se when administered alone. All procedures, doses and administration routes of the various drugs were chosen on the basis of previous studies [3,4,12,19,23,36] or in preliminary experiments carried out in our laboratory (data not shown).

Results are presented as mean ± S.E.M., except the ID_{50} values (i.e. the dose of myricitrin that reduce the nociceptive response by 50% relative to the control values), which are reported as geometric means accompanied by their respective 95% confidence limits. The ID_{50} values were determined by nonlinear regression from individual experiments using GraphPad software (GraphPad software, San Diego, CA, USA). The percentages of inhibition were calculated for the most effective dose used. Data concerning nociception to chemical stimuli were analyzed statistically using one-way ANOVA followed by Newman–Keuls' post hoc test. Statistical comparisons of mechanical and cold allodynia data were performed by two-way ANOVA, followed by Bonferroni's multi-comparison post hoc test. In all cases, differences were considered to be significant when $P < 0.05$.

Myricitrin (3–100 mg/kg, i.p.) produced dose-dependent inhibition (83 ± 2%) of bradykinin-induced nociception with mean ID_{50} of 12.4 (8.5–18.1) mg/kg (Fig. 1A). Post hoc comparisons with the Newman–Keuls test detected a significant difference with myricitrin treatment at doses of 10 mg/kg ($P < 0.01$), 30 mg/kg ($P < 0.001$) and 100 mg/kg ($P < 0.001$). Like bradykinin, i.pl. injection of prostaglandin E2 also induced nociceptive behaviors in mice. However, myricitrin had no effect on nociceptive response triggered by prostaglandin E2 ($P > 0.05$) (Fig. 1B).

As shown in Fig. 1C, only the highest dose of myricitrin (100 mg/kg, i.p.) inhibited the nociceptive behavior induced by cinnamaldehyde, with inhibition of 43 ± 10% ($P < 0.001$). Pretreatment of camphor (7.6 mg/kg, s.c.), a nonspecific TRP blocker, inhibited the cinnamaldehyde-induced nociception in 57 ± 8% ($P < 0.01$). Fig. 1D shows that myricitrin (1–100 mg/kg, i.p. 30 min before) also inhibited (71 ± 6%) the nociceptive response induced by acidified saline with the mean ID_{50} value of 22.0 (16.1–30.0) mg/kg. Post hoc comparisons with the Newman–Keuls test detected a significant difference with myricitrin at doses of 30 mg/kg ($P < 0.001$) and 100 mg/kg ($P < 0.01$). Moreover, the blocking of the acid-sensitive ion channels (ASICs) by amiloride (100 mg/kg, i.p.) also decreased the nociception mediated by acidified saline in 64 ± 5% ($P < 0.001$).

Intraplantar injection of menthol produced a marked nociception in mice. Previous treatment with the general TRP blocker ruthenium red (3 mg/kg, i.p.) reduced nociception evoked by menthol in 51 ± 7% ($P < 0.05$). Furthermore, myricitrin (1–30 mg/kg, i.p.) inhibited (95 ± 3%) menthol-induced nociceptive behavior with the mean ID_{50} value of 2.4 (1.5–3.7) mg/kg (Fig. 2A). Post hoc comparisons with the Newman–Keuls test detected significant differences with myricitrin treatment at doses of 10 mg/kg ($P < 0.001$) and 30 mg/kg ($P < 0.001$). A time-course analysis of the antinociceptive effect of myricitrin is shown in Fig. 2B. Myricitrin produced marked antinociception as early as 15 min after administration, an action that remained significant up to 60 min (Fig. 2B). Furthermore, menthol promoted mechanical and cold allodynia, lasting 2 and 4 h, respectively. Myricitrin (30 and 100 mg/kg, i.p.) reduced

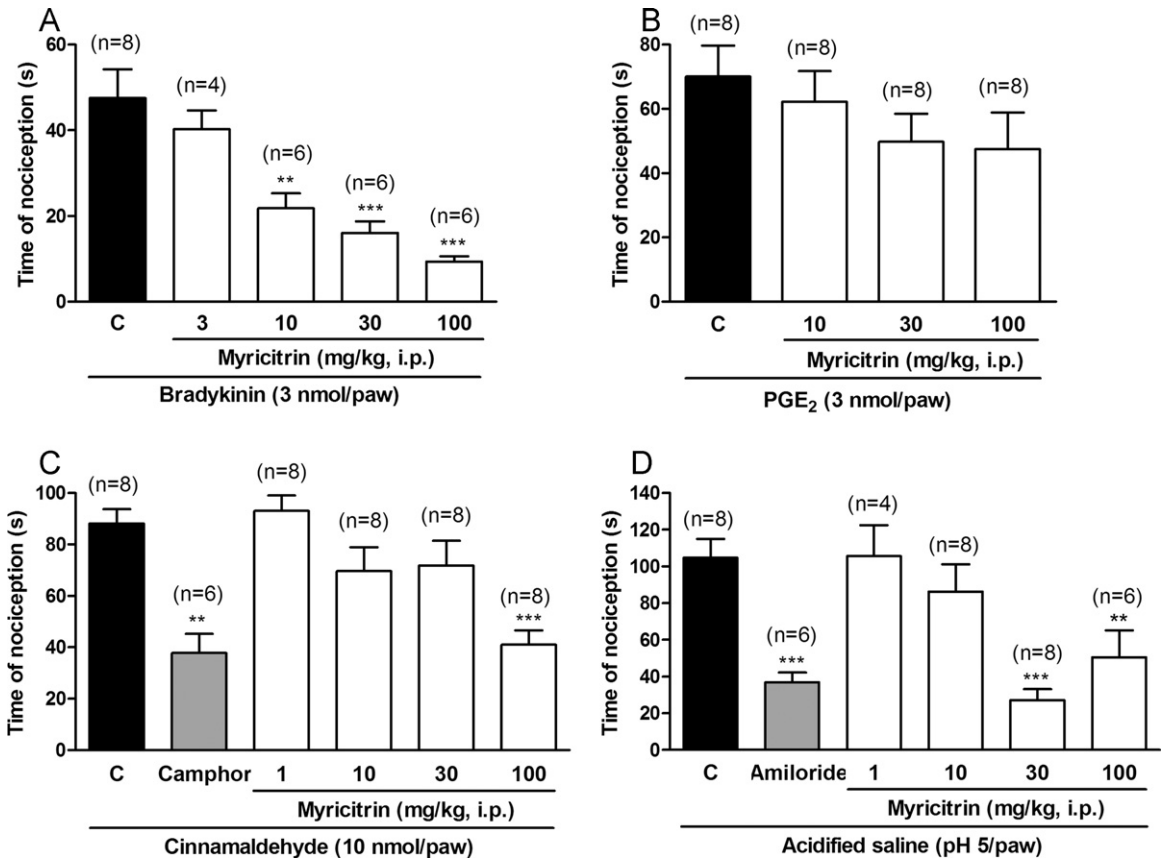

Fig. 1. Effect produced by intraperitoneal administration of myricitrin (3–100 mg/kg, i.p.) on bradykinin (3 nmol/paw, panel A)-, prostaglandin E_2 (3 nmol/paw, panel B)-, cinnamaldehyde (10 nmol/paw, panel C)- or acidified saline (pH 5/paw, panel D)-induced nociception in mice. Mice were treated with myricitrin, vehicle (C, 10 ml/kg; i.p.), camphor (7.6 mg/kg, s.c.) or amiloride (100 mg/kg, i.p.) 30 min prior to the i.pl. injection of respective algogens. Each column represents the mean ± S.E.M. of the number of animals (n) indicated in each group shown in the figure panels. Significance levels when compared to the control group are indicated by $^{**}P < 0.01$ and $^{***}P < 0.001$ (one way ANOVA followed by Newman–Keuls' test).

menthol-induced mechanical allodynia at 30 ($P < 0.05$ and 0.001), 60 ($P < 0.001$ and 0.01) and 120 ($P < 0.01$ for 100 mg/kg) min after myricitrin treatment (Fig. 2C). However, the highest dose of myricitrin (100 mg/kg) reduced menthol evoked cold allodynia at 60 min ($P < 0.05$) (Fig. 2D).

Studies have reported that flavonoids exert antioxidant, anti-inflammatory, immunomodulatory, antiallergic, neuroprotective, anti-mutagenic, anti-rheumatic and antinociceptive effects [8,9,25]. We have demonstrated that myricitrin reduced both acute and chronic pain in mice. The antinociceptive action of this flavonoid has been attributed to inhibition of protein kinase C activation, NO production and anti-inflammatory activity [2,14,25,26]. This study extends and confirms previous reports that myricitrin displays potent antinociceptive properties against nociception caused by bradykinin, acidified saline and TRPs channels agonists into the mouse hind paw. It is well established that bradykinin participates in the processes of nociceptor excitation and sensitization to other noxious or even innocuous stimuli through binding of specific G-protein-coupled kinin B2 receptors and by a direct activation of PKC- and an indirect activation of the PKA-signaling pathways [7,12]. In agreement with previous data [12], bradykinin injection into the hind paw caused a rapid and marked overt nociception in mice. The results shown here reveal that myricitrin abolished bradykinin-induced nociception. Additionally, it was reported that mechanical hyperalgesia induced by i.pl. injection of bradykinin in rats was reduced by myricitrin, an effect that seems to involve the activation of PKC [25]. Thus, the effect of the myricitrin against bradykinin-induced nociception and mechanical hyperalgesia might explain, at least partially, the ability of this flavonoid to interact with the PKC pathway.

Furthermore, bradykinin may also exert indirect effects on the nociceptive process, such as generating phospholipase A2 lipid mediators, namely prostaglandins and leukotrienes [7,38]. To date, prostaglandin E2-induced hind paw nociception in mice is mediated through activation of specific G-protein-coupled EP3 and EP4 receptors, with the involvement of both PKA and ERK signaling pathways [19]. However, treatment with myricitrin was ineffective in attenuating nociception (the current study) and the development of mechanical hyperalgesia [25] induced by prostaglandin E2 in mice and rats, respectively. Our data reinforce the notion that PKA-dependent pathway is probably not involved in the antinociception produced by myricitrin.

Moreover, there is evidence implicating the TRPV1 and TRPA1 activation in bradykinin-induced nociception through B2 receptors signaling in nociceptors [5,12]. Additionally, intraplantar injection of capsaicin and allyl isothiocyanate cause acute nociceptive behaviors in mice, an effect that is mediated via activation of TRPV1 and TRPA1 receptors, respectively, reinforcing the important role of its channels in acute nociception transmission [3,12]. It has been reported that myricitrin reduced nociception induced by intraplantar injection of capsaicin, an agonist of the TRPV1 receptor [25]. Remarkably, the present study demonstrates that myricitrin reduced the nociceptive response caused by intraplantar injection of cinnamaldehyde (an agonist of the TRPA1 receptor), with the same inhibition profile observed in neurogenic nociception caused by capsaicin.

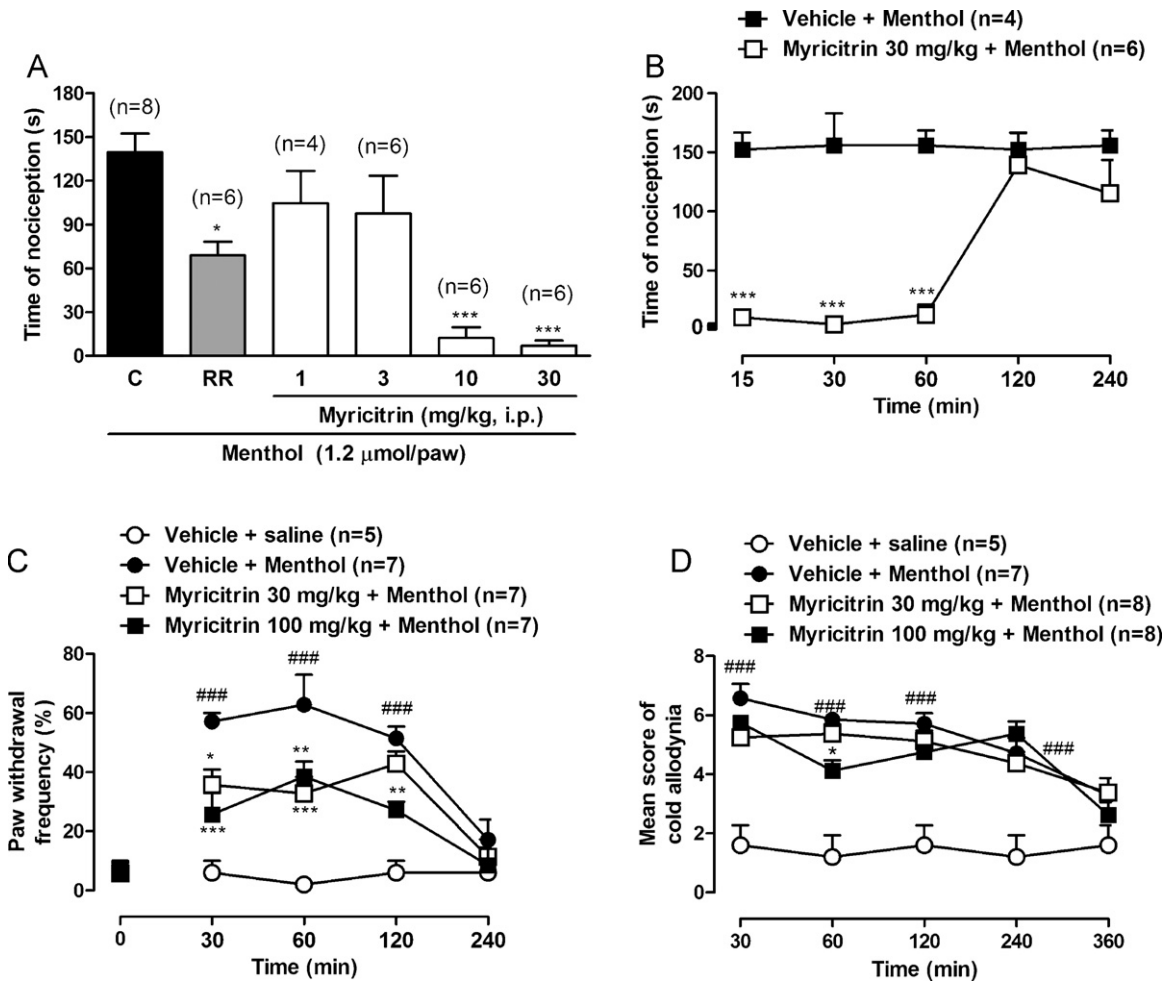

Fig. 2. Effect produced by intraperitoneal administration of myricitrin (1–100 mg/kg, i.p.) on nociception (panels A and B)-, mechanical (panel C)- and cold (panel D)-allodynia induced by intraplantar injection of menthol. Panel B shows the time-course of antinociceptive effect of myricitrin (30 mg/kg, i.p.) treatment. Mice received myricitrin, vehicle (C, 10 ml/kg, i.p.) or ruthenium red (RR, 3 mg/kg, i.p.) 30 min before menthol injection (1.2 μmol/paw). Each column represents the mean ± S.E.M. of the number of animals (*n*) indicated in each group shown in the figure panels and each point represents the mean ± S.E.M. of paw withdrawal frequency to von Frey hair stimulation or the nocifensive responses (cold score) evoked by acetone application to the ipsilateral hind paw at the times indicated thereafter. Significance levels when compared to the control groups are indicated by $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ (one way ANOVA followed by Newman–Keuls' test) and by $^{\#}P < 0.05$ when compared to the corresponding value of the vehicle + saline groups, $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ when compared to the corresponding value of the vehicle + menthol groups to cold and mechanical allodynia (two-way ANOVA followed by Bonferroni's test).

We also investigated the possible antinociceptive effect of myricitrin against nociception induced by intraplantar injection of acidified saline. Several studies demonstrated that protons promote direct activation of nociceptors [30,39] and hyperalgesia [37] and that TRPV1 and ASIC channels are proton-sensing ion channels [11,20]. In fact, tissue acidification down to pH 5 occurs in a variety of painful tissue pathologies and protons are thought to be involved in the generation of inflammatory pain [34]. Importantly, the nociceptive responses elicited by acidified saline were extensively inhibited by prior treatment with amiloride, a blocker of ASIC channels [40]. We have shown that previous treatment with myricitrin reduced acidified saline-induced nociception in mice. Thus, the antinociceptive effect of the myricitrin against acidified saline might be explained by the ability of this flavonoid to interact with ASIC or TRPV1 channels. However, we cannot exclude the modulation of other pH-sensing G-protein-coupled receptors in the antinociception promoted by myricitrin in acidosis-linked nociception [17].

Previous studies indicated that menthol (an active ingredient of peppermint) could lead to membrane depolarization and action potential firing on a subpopulation of primary afferent neurons through TRPM8 receptor activation [22,29,31]. Moreover, topical menthol produces an innocuous cooling sensation at low concentrations and a burning pain sensation at high concentrations [15,21,41]. Here, we also demonstrate that myricitrin attenuated the nociception, mechanical and cold allodynia induced by intraplantar injection of menthol. It should be pointed out that menthol may interact with other ion channels expressed in sensory neurons, and that the cold sensation appears to involve potassium channels in the transduction and modulation of temperature information [28,35]. In this regard, it is important to mention that myricitrin-induced antinociception also involves the opening of voltage- and small-conductance Ca^{2+}-gated K^{+} channels [24]. Another study showed that the flavonol myricetin (the non-glycosylated form of myricitrin) reduces the ongoing mechanical allodynia and heat hyperalgesia in response to the spinal nerve ligation in rats [16]. In addition, it has been demonstrated, in dorsal root ganglion cultures from normal animals, that myricetin also interferes with intracellular calcium concentration through inhibition of voltage activated Ca^{2+} channels in a PKC-dependent fashion, which strongly suggests that this related flavonoids could modulate other ion channels involved in painful conditions [16]. Nonetheless, the present data that myricitrin promotes antinociceptive and anti-allodynic actions after intraplantar injection of menthol could reflect a modulation of

Ca^{2+} and/or K^+ ion channels as well as an indirect or direct inhibition of the PKC signaling mechanisms.

Collectively, these findings confirm and largely extend results from literature by giving new insights about the complex antinociceptive profile of myricitrin. In addition to well known antinociceptive mechanisms previously demonstrated, myricitrin appears modulate the activation of nociceptors by bradykinin and TRPs agonist (i.e. cinnamaldehyde, acidified saline and menthol). Furthermore, myricitrin probably could prevent the sensitization process in peripheral nociceptors through inhibition of PKC signaling pathways. Together, it is reasonable to propose that myricitrin might be of potential interest in the development of new clinically relevant drugs for the management of pain.

Conflict of interest

None.

Acknowledgements

This work was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Financiadora de Estudos e Projetos [FINEP, Rede Instituto Brasileiro de Neurociência (IBN-Net)], Brazil. M.F.P. Werner and A.R.S. Santos are recipients of post-doctoral scholarship and research fellowship from the CNPq. Cordova, M.M. and Silva, M.D. thank CNPq and CAPES by fellowship support.

References

[1] P.K. Agrawal, Flavonoid Glycosides, Elsevier Science, New York, 1989.
[2] G. Agullo, L. Gamet-Payrastre, S. Manenti, C. Viala, C. Remesy, H. Chap, B. Payrastre, Relationship between flavonoid structure and inhibition of phosphatidylinositol 3-kinase: a comparison with tyrosine kinase and protein kinase C inhibition, Biochem. Pharmacol. 53 (1997) 1649–1657.
[3] E.L. Andrade, A.P. Luiz, J. Ferreira, J.B. Calixto, Pronociceptive response elicited by TRPA1 receptor activation in mice, Neuroscience 152 (2008) 511–520.
[4] S. Bang, K.Y. Kim, S. Yoo, Y.G. Kim, S.W. Hwang, Transient receptor potential A1 mediates acetaldehyde-evoked pain sensation, Eur. J. Neurosci. 26 (2007) 2516–2523.
[5] D.M. Bautista, S.E. Jordt, T. Nikai, P.R. Tsuruda, A.J. Read, J. Poblete, E.N. Yamoah, A.I. Basbaum, D. Julius, TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents, Cell 124 (2006) 1269–1282.
[6] L.B. Bortalanza, J. Ferreira, S.C. Hess, F. Delle Monache, R.A. Yunes, J.B. Calixto, Anti-allodynic action of the tormentic acid, a triterpene isolated from plant, against neuropathic and inflammatory persistent pain in mice, Eur. J. Pharmacol. 453 (2002) 203–208.
[7] J.B Calixto, D.A. Cabrini, J. Ferreira, M.M. Campos, Inflammatory pain: kinins and antagonists, Curr. Opin. Anaesthesiol. 14 (2001) 519–526.
[8] J.B. Calixto, M.M. Campos, M.F. Otuki, A.R.S. Santos, Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules, Planta Med. 70 (2004) 93–103.
[9] J.B. Calixto, M.F. Otuki, A.R.S. Santos, Anti-inflammatory compounds of plant origin. Part I. Action on arachidonic acid pathway, nitric oxide and nuclear factor kappa B (NF-kappaB), Planta Med. 69 (2003) 973–983.
[10] M.J. Caterina, D. Julius, Sense and specificity: a molecular identity for nociceptors, Curr. Opin. Neurobiol. 9 (1999) 525–530.
[11] M.J. Caterina, D. Julius, The vanilloid receptor: a molecular gateway to the pain pathway, Annu. Rev. Neurosci. 24 (2001) 487–517.
[12] J. Ferreira, G.L. da Silva, J.B. Calixto, Contribution of vanilloid receptors to the overt nociception induced by B2 kinin receptor activation in mice, Br. J. Pharmacol. 141 (2004) 787–794.
[13] S.J. Flatters, G.J. Bennett, Ethosuximide reverses paclitaxel- and vincristine-induced painful peripheral neuropathy, Pain 109 (2004) 150–161.
[14] L. Gamet-Payrastre, S. Manenti, M.P. Gratacap, J. Tulliez, H. Chap, B. Payrastre, Flavonoids and the inhibition of PKC and PI 3-kinase, Gen. Pharmacol. 32 (1999) 279–286.
[15] B.G. Green, The sensory effects of l-menthol on human skin, Somatosens. Mot. Res. 9 (1992) 235–244.
[16] T. Hagenacker, I. Hillebrand, A. Wissmann, D. Busselberg, M. Schafers, Anti-allodynic effect of the flavonoid myricetin in a rat model of neuropathic pain: Involvement of p38 and protein kinase C mediated modulation of Ca(2+) channels, Eur. J. Pain 14 (2010) 992–998.
[17] C.W. Huang, J.N. Tzeng, Y.J. Chen, W.F. Tsai, C.C. Chen, W.H. Sun, Nociceptors of dorsal root ganglion express proton-sensing G-protein-coupled receptors, Mol. Cell. Neurosci. 36 (2007) 195–210.
[18] D. Julius, A.I. Basbaum, Molecular mechanisms of nociception, Nature 413 (2001) 203–210.
[19] C.A. Kassuya, J. Ferreira, R.F. Claudino, J.B. Calixto, Intraplantar PGE2 causes nociceptive behaviour and mechanical allodynia: the role of prostanoid E receptors and protein kinases, Br. J. Pharmacol. 150 (2007) 727–737.
[20] O. Krishtal, The ASICs: signaling molecules? Modulators? Trends Neurosci. 26 (2003) 477–483.
[21] L.J. Macpherson, S.W. Hwang, T. Miyamoto, A.E. Dubin, A. Patapoutian, G.M. Story, More than cool: promiscuous relationships of menthol and other sensory compounds, Mol. Cell. Neurosci. 32 (2006) 335–343.
[22] D.D. McKemy, W.M. Neuhausser, D. Julius, Identification of a cold receptor reveals a general role for TRP channels in thermosensation, Nature 416 (2002) 52–58.
[23] F.C. Meotti, S. Coelho Idos, A.R.S. Santos, The nociception induced by glutamate in mice is potentiated by protons released into the solution, J. Pain 11 (2010) 570–578.
[24] F.C. Meotti, R. Fachinetto, L.C. Maffi, F.C. Missau, M.G. Pizzolatti, J.B. Rocha, A.R.S. Santos, Antinociceptive action of myricitrin: involvement of the K^+ and Ca^{2+} channels, Eur. J. Pharmacol. 567 (2007) 198–205.
[25] F.C. Meotti, A.P. Luiz, M.G. Pizzolatti, C.A. Kassuya, J.B. Calixto, A.R. Santos, Analysis of the antinociceptive effect of the flavonoid myricitrin: evidence for a role of the L-arginine–nitric oxide and protein kinase C pathways, J. Pharmacol. Exp. Ther. 316 (2006) 789–796.
[26] F.C. Meotti, F.C. Missau, J. Ferreira, M.G. Pizzolatti, C. Mizuzaki, C.W. Nogueira, A.R.S. Santos, Anti-allodynic property of flavonoid myricitrin in models of persistent inflammatory and neuropathic pain in mice, Biochem. Pharmacol. 72 (2006) 1707–1713.
[27] F.C. Meotti, T. Posser, F.C. Missau, M.G. Pizzolatti, R.B. Leal, A.R.S. Santos, Involvement of p38MAPK on the antinociceptive action of myricitrin in mice, Biochem. Pharmacol. 74 (2007) 924–931.
[28] J. Noel, K. Zimmermann, J. Busserolles, E. Deval, A. Alloui, S. Diochot, N. Guy, M. Borsotto, P. Reeh, A. Eschalier, M. Lazdunski, The mechano-activated K^+ channels TRAAK and TREK-1 control both warm and cold perception, EMBO J. 28 (2009) 1308–1318.
[29] M. Okazawa, T. Terauchi, T. Shiraki, K. Matsumura, S. Kobayashi, l-Menthol-induced $[Ca^{2+}]i$ increase and impulses in cultured sensory neurons, Neuroreport 11 (2000) 2151–2155.
[30] M. Omori, M. Yokoyama, Y. Matsuoka, H. Kobayashi, S. Mizobuchi, Y. Itano, K. Morita, H. Ichikawa, Effects of selective spinal nerve ligation on acetic acid-induced nociceptive responses and ASIC3 immunoreactivity in the rat dorsal root ganglion, Brain Res. 1219 (2008) 26–31.
[31] A.M. Peier, A. Moqrich, A.C. Hergarden, A.J. Reeve, D.A. Andersson, G.M. Story, T.J. Earley, I. Dragoni, P. McIntyre, S. Bevan, A. Patapoutian, A TRP channel that senses cold stimuli and menthol, Cell 108 (2002) 705–715.
[32] L.S. Premkumar, G.P. Ahern, Induction of vanilloid receptor channel activity by protein kinase C, Nature 408 (2000) 985–990.
[33] L.S. Premkumar, M. Raisinghani, S.C. Pingle, C. Long, F. Pimentel, Downregulation of transient receptor potential melastatin 8 by protein kinase C-mediated dephosphorylation, J. Neurosci. 25 (2005) 11322–11329.
[34] P.W. Reeh, M. Kress, Molecular physiology of proton transduction in nociceptors, Curr. Opin. Pharmacol. 1 (2001) 45–51.
[35] G. Reid, M. Flonta, Cold transduction by inhibition of a background potassium conductance in rat primary sensory neurones, Neurosci. Lett. 297 (2001) 171–174.
[36] A.R.S. Santos, J.B. Calixto, Ruthenium red and capsazepine antinociceptive effect in formalin and capsaicin models of pain in mice, Neurosci. Lett. 235 (1997) 73–76.
[37] N.K. Sharma, J.M. Ryals, H. Liu, W. Liu, D.E. Wright, Acidic saline-induced primary and secondary mechanical hyperalgesia in mice, J. Pain (2009).
[38] J. Shin, H. Cho, S.W. Hwang, J. Jung, C.Y. Shin, S.Y. Lee, S.H. Kim, M.G. Lee, Y.H. Choi, J. Kim, N.A. Haber, D.B. Reichling, S. Khasar, J.D. Levine, U. Oh, Bradykinin-12-lipoxygenase-VR1 signaling pathway for inflammatory hyperalgesia, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 10150–10155.
[39] K.H. Steen, P.W. Reeh, F. Anton, H.O. Handwerker, Protons selectively induce lasting excitation and sensitization to mechanical stimulation of nociceptors in rat skin, in vitro, J. Neurosci. 12 (1992) 86–95.
[40] R Waldmann, G. Champigny, F. Bassilana, C. Heurteaux, M. Lazdunski, A proton-gated cation channel involved in acid-sensing, Nature 386 (1997) 173–177.
[41] G. Wasner, J. Schattschneider, A. Binder, R. Baron, Topical menthol—a human model for cold pain by activation and sensitization of C nociceptors, Brain 127 (2004) 1159–1171.
[42] M. Zimmermann, Ethical guidelines for investigations of experimental pain in conscious animals, Pain 16 (1983) 109–110.