Pharmacology, Biochemistry and Behavior 99 (2011) 782–788

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Antinociceptive and anti-inflammatory activities of nicotinamide and its isomers in different experimental models

Adriana M. Godin, Wallace C. Ferreira, Leonardo Tadeu S. Rocha, João Gabriel T. Seniuk, André Luiz L. Paiva, Leonardo A. Merlo, Elias B. Nascimento Jr., Leandro F.S. Bastos, Márcio M. Coelho *

Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Avenida Antônio Carlos 6627, 31270-901, Belo Horizonte, MG, Brazil

ARTICLE INFO

Article history:
Received 27 May 2011
Received in revised form 30 June 2011
Accepted 4 July 2011
Available online 8 July 2011

Keywords:
Nicotinamide
Picolinamide
Isonicotinamide
Inflammation
Pain
Nociception
Nociceptive behavior
Edema

ABSTRACT

Although there is evidence for the anti-inflammatory activity of nicotinamide, there is no evaluation of its effects in models of nociceptive and inflammatory pain. In addition, there is no information about the potential anti-inflammatory and antinociceptive activities of the nicotinamide isomers, picolinamide and isonicotinamide. Per os (p.o.) administration of nicotinamide (1000 mg/kg, −1 h) inhibited the first and second phases of the nociceptive response induced by formalin in mice. In the model of nociceptive pain, exposure of mice to a hot-plate (50 °C), nicotinamide (1000 mg/kg, −1 h) also presented antinociceptive activity. Nicotinamide (500 mg/kg, −1 and 3 h) also inhibited the mechanical allodynia induced by carrageenan in rats, a model of inflammatory pain. In addition to inhibiting the nociceptive response, nicotinamide (500 or 1000 mg/kg, −1 and 3 h) inhibited the paw edema induced by carrageenan in mice and rats. P.o. administration of picolinamide (125 mg/kg, −1 h) and isonicotinamide (500 or 1000 mg/kg, −1 h) inhibited the second phase of the nociceptive response induced by formalin in mice. The paw edema induced by carrageenan in mice was also inhibited by isonicotinamide (500 or 1000 mg/kg, −1 h) and picolinamide (125 mg/kg, −1 h and 3 h). The results represent the first demonstration of the activity of nicotinamide and its isomers in models of nociceptive and inflammatory pain and provide support to their anti-inflammatory activity. The demonstration of new activities for nicotinamide is important as it may contribute to expand its use in the treatment of other pathological conditions.

1. Introduction

Nicotinamide is useful to prevent or treat pellagra, a disease characterized by diarrhea, dementia and dermatitis (Hegyi et al., 2004). However, there are many studies clearly showing that nicotinamide also has other functions. It has been shown to protect rat pancreatic beta cells exposed to streptozotocin or hydrogen peroxide (Hoorens and Pipeleers, 1999) and prevent streptozotocin-induced diabetes in rats (Junod et al., 1969). Some studies have also demonstrated the anti-inflammatory activity of nicotinamide in experimental models of arthritis induced by potassium peroxochromate in mice (Miesel et al., 1995) and pleurisy induced by carrageenan in rats (Cuzzocrea et al., 1999b). The efficacy of nicotinamide in models of traumatic brain injury was also reported (Hoane et al., 2003, 2006a, 2006b). In addition to its anti-inflammatory activity in experimental models, nicotinamide has also been shown to induce beneficial effects in patients with rheumatoid arthritis (Hoffer, 1959).

Although the mechanisms that contribute to these activities of nicotinamide are not clear, it is possible that inhibition of poly(ADP-ribose) polymerase-1 (PARP-1) may have an important role (Radons et al., 1994; Uchigata et al., 1982; Virág and Szabó, 2002). This enzyme transfers adenosine diphosphate-ribose units from nicotinamide adenine dinucleotide (NAD^+) to many nuclear proteins. Although such activity is important to many processes including gene expression, DNA repair and apoptosis, excessive activation of PARP-1 is implicated in the development of inflammatory conditions (Hassa and Hottiger, 2002; Szabó, 1998). Excessive activation of PARP-1 may result in marked reduction of NAD^+ content, leading to energy depletion, dysfunction and cell death (Giansanti et al., 2010; Szabó, 1998). Direct evidence supporting the inflammatory role of PARP-1 has been derived from studies in which a reduced inflammatory response was observed in animals deficient of this enzyme or treated with pharmacological inhibitors (Cuzzocrea et al., 1999a, 1999b; Szabó, 1998).

Nicotinamide, a PARP-1 inhibitor and a precursor of NAD^+, maintains the concentrations of this coenzyme and may prevent cellular alterations resulting from excessive activation of PARP-1 (Uchigata et al., 1982). Although there is evidence for the anti-inflammatory activity of the nicotinamide, derived mainly from in vitro studies, few studies investigated its effect in in vivo models of inflammation (Cuzzocrea et al., 1999a, 1999b; Miesel et al., 1995). Regarding the effect in experimental models of pain, nicotinamide reverses the mechanical and thermal hyperalgesia in streptozotocin

* Corresponding author. Tel.: +55 031 3409 6965.
E-mail address: mmcoelho@farmacia.ufmg.br (M.M. Coelho).

0091-3057/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2011.07.003

diabetic rats (Stevens et al., 2007). To the best of our knowledge, the effect induced by nicotinamide in models of nociceptive and inflammatory pain has not been investigated. In addition, there is no information about the potential anti-inflammatory and antinociceptive activities of its isomers, picolinamide and isonicotinamide, although both inhibit the activity of PARP-1 (Griffin et al., 1995) and the late increases the activity of silent information regulator 2 (Sauve et al., 2005), a NAD^+-dependent histone deacetylase that has been suggested to present both anti- and pro-inflammatory activities (Gallí et al., 2011).

Thus, we aimed to investigate the effects induced by nicotinamide in experimental models of nociceptive and inflammatory pain and inflammatory edema, in order to provide further information that may contribute to expand its use in the treatment of painful or inflammatory conditions. The rationale to investigate the effects induced by the two nicotinamide isomers, picolinamide and isonicotinamide (Fig. 1), is underscored by the fact that both also inhibit PARP-1 (Itoh et al., 1984; Uchigata et al., 1982) and there is not any information about their potential anti-inflammatory and antinociceptive activities.

2. Materials and methods

2.1. Animals

Female Swiss mice (24–28 g) and female Wistar rats (200–250 g) were used. The animals had free access to food and water and were maintained in a room with a 12 h light-dark cycle for at least 3 days before the experiment to allow acclimatization. The experiments were carried out at a room temperature of 27 °C. This temperature was used because the thermoneutral zone for mice and rats ranges between 26 and 34 °C, a temperature range that markedly differs from that of standard laboratory environments which could be stressful and affect many aspects of physiology and behavior (Gaskill et al., 2009; Gordon, 1990). All experiments were conducted according to the ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann, 1983) and approved by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais.

2.2. Drugs

Nicotinamide (Sigma, USA), isonicotinamide (Sigma-Aldrich, France), picolinamide (Sigma-Aldrich, Germany), λ-carrageenan (Sigma, USA), formaldehyde 37% m/v (Sigma, USA), dipyrone (Sanofi Aventis, Brazil), phenobarbital (Aventis Pharma, Brasil) and carboxymethylcellulose sodium salt (Sigma, USA) were used. The suspensions were prepared in 0.5% of carboxymethylcellulose sodium salt suspension in saline immediately before the experiments. The volume of per os (p.o.) administration was 12 ml/kg either for mice or rats.

2.3. Evaluation of the nociceptive response induced by formalin in mice

Formalin (2.5%, 20 μl) was injected via the subcutaneous (s.c.) route into the dorsum of the right hind paw of mice 1 h after p.o. administration of nicotinamide, isonicotinamide or picolinamide. Each mouse was placed under a transparent glass funnel (18 cm diameter, 15 cm-high) and the amount of time that the animal licked the injected paw was monitored between 0 and 5 min (first phase) and 15 and 30 min (second phase) after the injection of formalin.

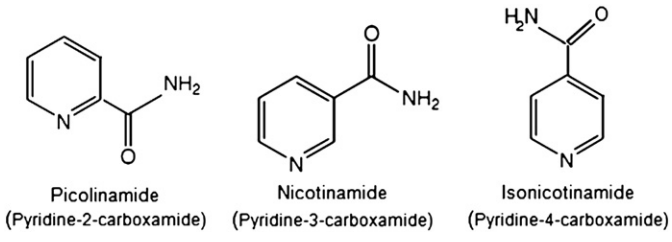

Fig. 1. Chemical structures of nicotinamide and its isomers, picolinamide and isonicotinamide.

2.4. Evaluation of the nociceptive response of mice in the hot-plate model

A preliminary protocol was carried out to evaluate the latency for the nociceptive behavior after exposing the mice on plate temperatures of 50°, 52°, 54° or 56 °C. We selected the temperatures of 50 °C (latency ≈ 15 s) or 54 °C (latency ≈ 8 s) to evaluate the effect induced by nicotinamide in this model of nociceptive pain. One hour after treatment with nicotinamide or dipyrone (positive control), each animal was placed on the heated (50 or 54 °C) metal plate. The latency to lick one of the hind paws or to jump off plate was determined. The animal was removed from the hot-plate immediately after the response. The cut-off times were 30 or 50 s (temperatures of 54 or 50 °C, respectively) to avoid tissue damage.

2.5. Evaluation of the edema induced by carrageenan in rats or mice

Paw edema was measured with a plethysmometer (Model 7140, Ugo Basile, Italy). The basal volume of the right hind paw was measured before administration of any drug. After determination of the basal volume, the animals were divided in the experimental groups in such a way that the mean volumes of the different groups were similar. Carrageenan was injected via the intraplantar (i.pl.) route in rats (500 μg, 50 μl) or mice (600 μg, 30 μl). Considering that (a) the paw volume was measured at 2, 4 and 6 h later, (b) the half-life of nicotinamide may vary between 1 h (mice; Rojas et al., 1993) and 5 h (rats; Petrack et al., 1966) and (c) preliminary results indicated that only one dose of nicotinamide did not markedly inhibit the response evaluated, we administered two doses of nicotinamide, 1 h before and 3 h after carrageenan. Similar protocol was used for picolinamide. However, for isonicotinamide, only one dose, 1 h before carrageenan, was used, as this was sufficient to demonstrate the investigated effect. The results were presented as the paw volume variation in relation to the basal values.

2.6. Evaluation of the mechanical allodynia induced by carrageenan in rats

Mechanical allodynia was measured with a 40 mN nylon filament (Sorri, Brazil) as previously described (Souza et al., 2002). Briefly, the rats were kept individually in Perspex boxes (20 × 20 cm with 18 cm-high walls) whose floor was a metal grid through which the filament was pressed on the plantar surface of the right hind paw with the strength just necessary to cause it to bend for approximately 1 s. The number of withdrawal reflexes was determined in a trial of 10 touches for each rat. The basal withdrawal frequency was determined before administration of any drug. After determination of the basal withdrawal frequency, the animals were divided in the experimental groups in such a way that the mean withdrawal frequencies of the different groups were similar. The withdrawal frequency was measured at different times after injection of the inflammatory stimulus. Nicotinamide was administered 1 h before and 3 h after carrageenan.

2.7. Evaluation of the motor activity of mice

The motor activity of the animals was evaluated in a rota-rod apparatus. The animals were trained on the apparatus for 3 days before the experiment. On the experimental day, the animals were placed on a rota-rod (14 rpm) and the time they spent on it was measured. The cut-off time was 2 min. After determination of the baseline values, the animals were treated with nicotinamide, isonicotinamide, picolinamide

or phenobarbital (positive control) and 1 h later they were again tested in the apparatus. In another protocol, we evaluated the effect induced by two administrations of nicotinamide to validate the results observed in the model of mechanical allodynia. The first administration occurred 3 h before the first evaluation of the motor activity. The second administration occurred 1 h after the first evaluation of the motor activity. After the second administration, the motor activity was evaluated at 1 and 3 h.

2.8. Data collection and statistical analysis

Two observers evaluated the nociceptive behavior and edema in the different experimental protocols. The observers were not aware of the treatments and registered the nociceptive behavior or edema of animals from the different experimental groups within each protocol. The results, presented as mean ± standard error mean (S.E.M.), were analyzed by one-way analysis of variance followed by Newman–Keuls post-hoc test when the main effect was significant. A $P<0.05$ was considered significant.

3. Results

Nicotinamide, at the doses of 125 or 250 mg/kg, did not inhibit the nociceptive response induced by formalin in mice (data not shown). However, in another protocol in which the doses of 500 or 1000 mg/kg were used, we observed that the highest dose inhibited both phases of nociceptive response (Fig. 2). Nicotinamide (1000 mg/kg) also increased the latency for the nociceptive response in the hot-plate model when a thermal stimulus of lower intensity (50 °C) was used (Fig. 3). However, when a thermal stimulus of higher intensity (54 °C) was used, the antinociceptive effect was not observed (Fig. 4). In both cases, an increase of the latency for the nociceptive response was observed after treatment of the animals with dipyrone (500 mg/kg). Nicotinamide (500 or 1000 mg/kg) also inhibited the mechanical allodynia induced by carrageenan in rats (Fig. 5).

To validate the results observed in the nociceptive models, we investigated the effects induced by nicotinamide on the motor coordination of the animals. A single dose of nicotinamide (1000 mg/kg, −1 h) did not alter the time mice spent in the rota-rod apparatus. As mentioned in the Materials and methods section, we also evaluated the effect induced by two administrations of nicotinamide (500 or 1000 mg/kg). The first administration occurred 3 h before and the second administration occurred 1 h after the first evaluation of the motor activity. After the second administration, the motor activity was evaluated at 1 and 3 h. In this protocol, we observed that while the lowest dose did not alter the motor coordination of the animals, the highest dose partially reduced this response 1 h after the second administration. Such results validate the antinociceptive effect induced by the lowest dose of nicotinamide. The positive control, phenobarbital (50 mg/kg) markedly inhibited the performance of the animals (Table 1).

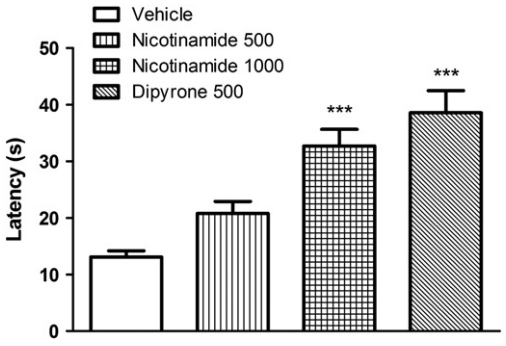

Fig. 3. Effect induced by nicotinamide (500 or 1000 mg/kg, p.o., −1 h) or dipyrone (500 mg/kg, p.o., −1 h) on the nociceptive response induced by heat (hot plate model, 50 °C) in mice. *** significantly different from vehicle ($P<0.001$). n = 10.

In addition to inhibiting the nociceptive response, nicotinamide (1000 mg/kg) also inhibited the paw edema induced by carrageenan in mice (Fig. 6). Aiming to verify whether this inhibition also occurs in other species, we observed that nicotinamide (1000 mg/kg) also inhibits the inflammatory edema in rats (Fig. 7).

Next, we investigated whether the nicotinamide isomers, isonicotinamide and picolinamide, also present similar activities. Isonicotinamide (500 or 1000 mg/kg) inhibited the second phase of nociceptive response induced by formalin in mice (Fig. 8). However, when we evaluated the effects induced by picolinamide (500 or 1000 mg/kg), we observed lethality. Therefore, the doses were reduced to 62.5 or 125 mg/kg. Information in the literature about the lethal dose of the picolinamide was not found. Picolinamide (125 mg/kg), inhibited the second phase of nociceptive response induced by formalin in mice (Fig. 9).

To validate the results observed in the nociceptive models, we investigated the effects induced by the nicotinamide isomers on the motor coordination of mice. Isonicotinamide (500 or 1000 mg/kg) or picolinamide (62.5 or 125 mg/kg) did not alter the time mice spent in the rota-rod apparatus (Table 1).

In addition to inhibiting the nociceptive response, isonicotinamide and picolinamide induced an anti-inflammatory effect in mice. Isonicotinamide (1000 mg/kg), administered 1 h before carrageenan, inhibited the paw edema at 2, 4 and 6 h (Fig. 10). Picolinamide (125 mg/kg), administered 1 h before and 3 h after carrageenan, inhibited the paw edema only at 6 h (Fig. 11).

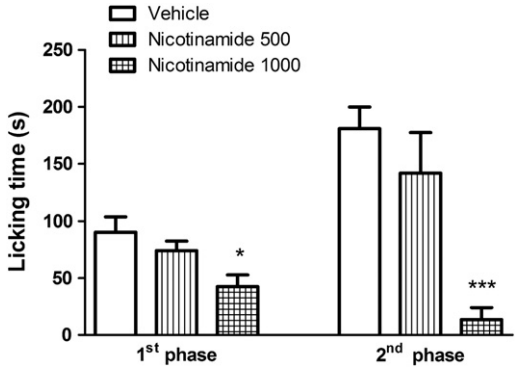

Fig. 2. Effect induced by nicotinamide (500 or 1000 mg/kg, p.o., −1 h) on the nociceptive response induced by formalin (2.5%; 20 µl; s.c.) in mice. * and *** significantly different from vehicle ($P<0.05$ and $P<0.001$, respectively). n = 8.

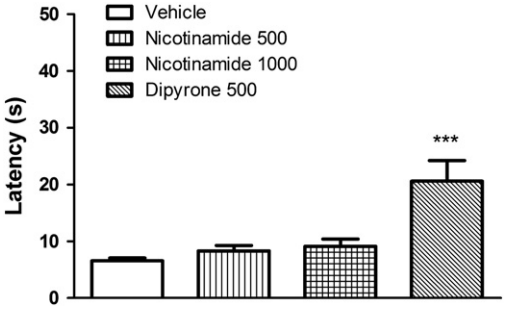

Fig. 4. Effect induced by nicotinamide (500 or 1000 mg/kg, p.o., −1 h) or dipyrone (500 mg/kg, p.o., −1 h) on the nociceptive response induced by heat (hot plate model, 54 °C) in mice. *** significantly different from vehicle ($P<0.001$). n = 10.

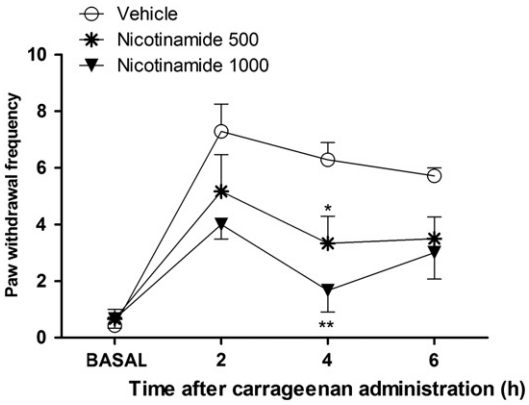

Fig. 5. Effect induced by nicotinamide (500 or 1000 mg/kg, p.o., −1 h and 3 h) on the mechanical allodynia induced by carrageenan (500 μg, 50 μl, i.pl.) in rats. * and ** significantly different from vehicle ($P<0.05$ and $P<0.01$, respectively). n = 8.

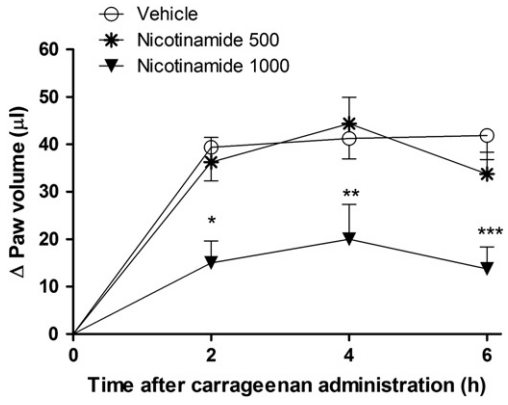

Fig. 6. Effect induced by nicotinamide (500 or 1000 mg/kg, p.o., −1 h and 3 h) on the edema induced by carrageenan in mice. The basal paw volumes of the groups treated with vehicle, nicotinamide 500 and nicotinamide 1000 were 102 ± 4, 102 ± 2 and 103 ± 3 μl, respectively. *, ** and *** significantly different from vehicle ($P<0.05$, $P<0.01$ and $P<0.001$, respectively). n = 8.

4. Discussion

We initially observed that nicotinamide inhibited the nociceptive response induced by formalin, an experimental model of nociceptive and inflammatory pain. The first phase, that starts immediately after the injection of formalin, has been attributed to direct activation by formaldehyde of transient receptor potential (TRP) channels, specifically TRPA1 (McNamara et al., 2007) and TRPV1 (Tian et al., 2009), in nociceptors. The late phase starts approximately 15 min after formalin injection and is associated with the development of an inflammatory response at the injection site that contributes to the activation or sensitization of nociceptors and changes in the excitability of dorsal horn neurons (Tjolsen et al., 1992). Although nicotinamide inhibited both phases of the nociceptive response, the second phase was inhibited to a greater extent indicating a profile similar to that of anti-inflammatory drugs. It is well established that anti-inflammatory drugs markedly inhibit the second phase of the nociceptive response induced by formalin, while exert little or no effect on the first phase (Hunskaar and Hole, 1987). It has been shown that nicotinamide inhibits the production of cytokines (Ungerstedt et al., 2003), prostaglandin (PG) E_2 (Cuzzocrea et al., 1999b) and nitric oxide (NO) (Kao et al., 2007), inflammatory mediators that may contribute to the second phase of the nociceptive response induced by formalin. Whether the inhibitory effect induced by nicotinamide on the production of inflammatory mediators results from inhibition of PARP-1 is not known. However, a connection between the antinociceptive effect induced by nicotinamide in the formalin model with PARP-1 inhibition is attractive as it has been recently demonstrated that formaldehyde activates this enzyme in cell cultures (Lim et al., 2010).

Nicotinamide also inhibited the mechanical allodynia and edema induced by carrageenan. These responses result from the action of many inflammatory mediators such as NO (Handy and Moore, 1998; Nakamura et al., 1996), eicosanoids (Tonussi and Ferreira, 1994; Vinegar et al., 1987), and cytokines (Chen et al., 1994; Cunha et al., 2000), among others. Moreover, local neutrophil infiltration and activation also contribute to the response induced by carrageenan (Salvemini et al., 1996) by producing chemokines, cytokines, NO and reactive oxygen species. As it has been demonstrated that carrageenan increases the staining of PARP-1 (Cuzzocrea et al., 2004) and nicotinamide inhibits the activity of this enzyme (Radons et al., 1994; Uchigata et al., 1982), it is tempting to associate this last effect with the inhibition of carrageenan-induced responses. It has been shown that PARP-1 inhibitors, including nicotinamide, may inhibit the production of many inflammatory mediators that contribute to the mechanical allodynia and edema induced by carrageenan. Cuzzocrea et al. (1999b) demonstrated that two PARP-1 inhibitors, nicotinamide and 3-aminobenzamide, inhibit the production of PGE_2 and leukocyte migration in a model of pleurisy induced by carrageenan. Some studies demonstrated that nicotinamide decreases the activation of NF-kB (Crowley et al., 2000; Grange et al., 2009; Pero et al., 1999) and the production of inflammatory cytokines such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α (Fukuzawa

Table 1
Effect induced by nicotinamide (500 or 1000 mg/kg, p.o., −1 h or −1 h and 3 h), isonicotinamide (1000 mg/kg, p.o., −1 h), picolinamide (125 mg/kg, p.o., −1 h) or phenobarbital (50 mg/kg, p.o., −1 h) on the time spent by mice on the rotating rod.

Treatment	Time spent on the rotating rod (s)			
	Baseline	1 h		
Vehicle	118 ± 2	119 ± 1		
Nicotinamide 1000	120 ± 0	120 ± 0		
Isonicotinamide 1000	120 ± 0	120 ± 0		
Picolinamide 125	118 ± 2	120 ± 0		
Phenobarbital 50	120 ± 0	15 ± 4[a]		
	Baseline	2 h	4 h	6 h
Vehicle	120 ± 0	120 ± 0	120 ± 0	120 ± 0
Nicotinamide 500	120 ± 0	120 ± 0	120 ± 0	120 ± 0
Nicotinamide 1000	120 ± 0	120 ± 0	66 ± 18[b]	95 ± 15

[a,b] significantly different from respective vehicle-treated group at the same time point ($P<0.001$). n = 8.

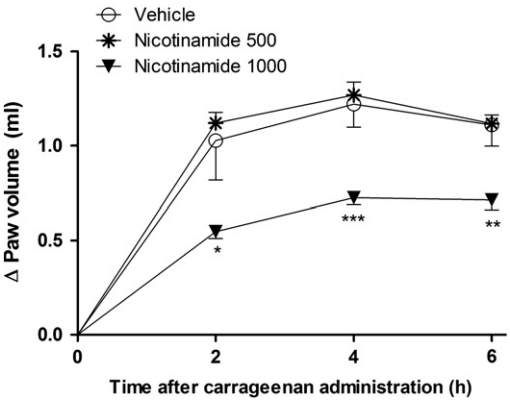

Fig. 7. Effect induced by nicotinamide (500 or 1000 mg/kg, p.o., −1 h and 3 h) on the edema induced by carrageenan in rats. The basal paw volumes of the groups treated with vehicle, nicotinamide 500 and nicotinamide 1000 were 1.16 ± 0.08, 1.16 ± 0.06 and 1.15 ± 0.04 ml, respectively. *, ** and *** significantly different from vehicle ($P<0.05$, $P<0.01$ and $P<0.001$, respectively). n = 6.

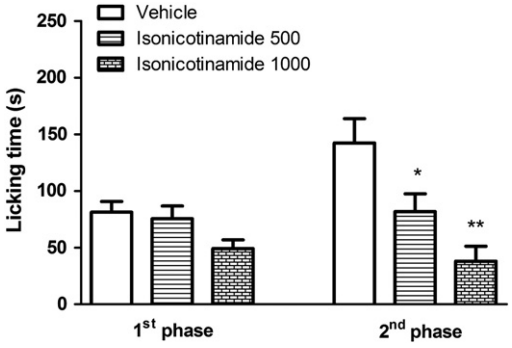

Fig. 8. Effect induced by isonicotinamide (500 or 1000 mg/kg, p.o., −1 h) on the nociceptive response induced by formalin (2.5%; 20 μl; s.c.) in mice. * and ** significantly different from vehicle ($P<0.05$ and $P<0.01$, respectively). n = 8.

et al., 1997), IL-6 and IL-8 (Ungerstedt et al., 2003) and also eicosanoids such as PGE_2 (Cuzzocrea et al., 1999b). Oliver et al. (1999) provided further support for the inflammatory role of PARP-1 by demonstrating that PARP-1-deficient mice have reduced expression of NF-kB, decreased production of cytokines and other inflammatory mediators such as NO, TNF-α and IFN-γ.

Although a marked inhibition of the second phase of the nociceptive response induced formalin and also the mechanical allodynia and edema induced by carrageenan in animals previously treated with nicotinamide is suggestive of an anti-inflammatory profile, this drug also inhibited the response in an experimental model of nociceptive pain induced by heat. As the nociceptive response in this model is immediate (few seconds), it is highly unlikely that the effect induced by nicotinamide results from inhibition of PARP-1 or reduced production of inflammatory mediators. Inhibition of such immediate response would likely be associated with direct inhibition of neuronal excitability. Although there is scarce data on the effect induced by nicotinamide on neuronal excitability, it has been recently demonstrated that NAD^+, which may be derived from nicotinamide (Ijichi et al., 1966), activates a class of sodium-activated potassium channels, Slack K_{Na} (Tamsett et al., 2009). These channels may have a role in nociceptors excitability as it has been suggested that they regulate dorsal root ganglion (DRG) neuronal resting membrane potential (Bischoff et al., 1998). Curiously, an anti-inflammatory drug with analgesic activity, niflumic acid, activates this class of K_{Na} channels (Dai et al., 2010). In addition, protein kinase A, an enzyme activated by many inflammatory mediators, induces the internalization of these channels and increases DRG neuron excitability (Nuwer et al., 2010). Modulation of TRPV1 and $Na_v1.8$ sodium channels, important targets in the nociceptive processing pathways (Jara-Oseguera et al., 2008; Mathie, 2010; Villareal et al., 2009), by nicotinamide and its isomers may also be indicated as a

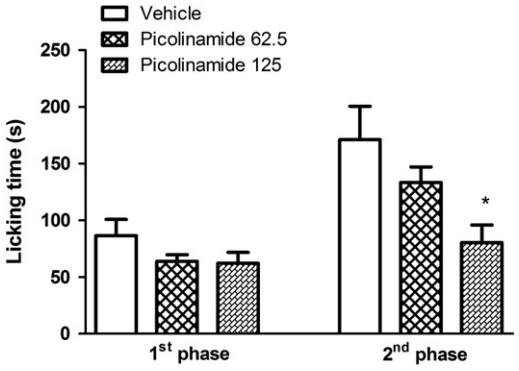

Fig. 9. Effect induced by picolinamide (62.5 or 125 mg/kg, p.o., −1 h) on the nociceptive response induced by formalin (2.5%; 20 μl; s.c.) in mice. * significantly different from vehicle ($P<0.05$). n = 8.

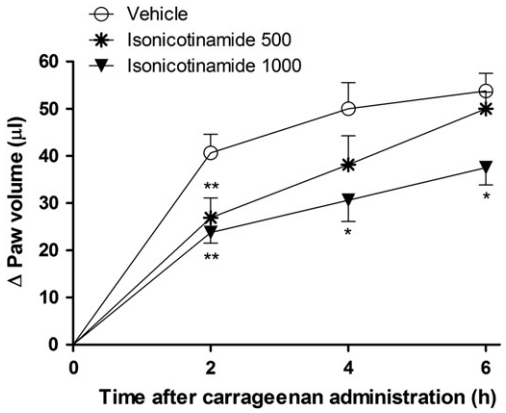

Fig. 10. Effect induced by isonicotinamide (500 or 1000 mg/kg, p.o., −1 h) on the edema induced by carrageenan in mice. The basal paw volumes of the groups treated with vehicle, isonicotinamide 500 and isonicotinamide 1000 were 89 ± 2, 88 ± 2 and 87 ± 2 μl, respectively. * and ** significantly different from vehicle ($P<0.05$ and $P<0.01$, respectively). n = 8.

possibility. It has been shown that some nicotinamide derivatives, although very distinct from the parent compound, act as TRPV1 (Westaway et al., 2008) or $Na_v1.8$ sodium channels (Kort et al., 2010) antagonists.

The effect induced by nicotinamide in the hot-plate model varied according to the intensity of the thermal stimulus as it increased the latency for the nociceptive response when the mice were exposed to a low (50 °C), but not to a high (54 °C) temperature. This profile has also been demonstrated for other drugs. The antinociceptive effect induced by some opioid analgesics, such as buprenorphine and dezocine, is observed at the temperature of 53° but not at 56 °C (Fischer et al., 2008). These results confirm the probability of demonstrating the antinociceptive activity of a drug in an experimental model of nociceptive pain when using a thermal stimulus of lower intensity (Plone et al., 1996). It can be hypothesized that temperatures of 50 and 54 °C activate different nociceptive mechanisms that are differently affected by many drugs, including opioids (Fischer et al., 2008) and nicotinamide. Providing support to this possibility, Caterina et al. (1997, 1999) have shown that temperatures above 43 °C activate TRPV1 channels in sensory neurons, while temperatures superior to 52 °C are necessary to activate TRPV2 channels.

The effect induced by nicotinamide on the performance of the animals on the rotating rod was also evaluated to determine whether the effects observed in the experimental models of pain represent a genuine antinociceptive effect of nicotinamide itself without confounding effect on motor coordination or muscle tone. This is important as it

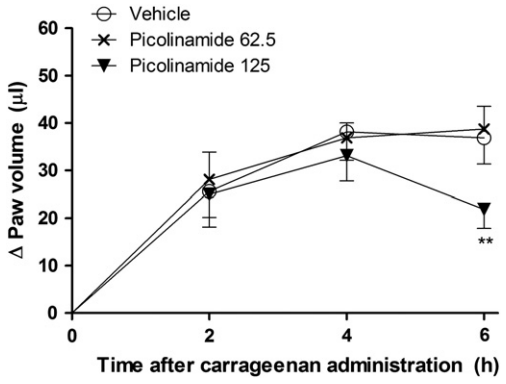

Fig. 11. Effect induced by picolinamide (62.5 or 125 mg/kg, p.o., -1 h and 3 h) on the edema induced by carrageenan in mice. The basal paw volumes of the groups treated with vehicle, picolinamide 62.5 and picolinamide 125 were 105 ± 3, 103 ± 4 and 105 ± 3 μl, respectively. ** significantly different from vehicle ($P<0.01$). n = 8.

has been demonstrated that nicotinamide inhibits seizures induced by pentylenetetrazole or kynurenine, suggesting a central depressant effect (Lapin, 1981). However, nicotinamide and its isomers, administered at single doses, did not alter the motor coordination of the animals. Only the highest dose (1000 mg/kg) of nicotinamide, administered twice 3 h apart, partially inhibited the motor coordination. Thus, it is unlikely that nicotinamide inhibition of the nociceptive behavior evaluated in the different experimental models is due to motor incoordination or a muscle relaxing effect.

Although we did not determine the plasma concentrations of nicotinamide in the experimental animals, it is possible that they correlate with the concentrations that inhibit the production of inflammatory mediators or enzyme activity in vitro. Ungerstedt et al. (2003) showed that concentrations of nicotinamide that inhibit cytokine production or PARP-1 activity in vitro vary from 2 to 40 mmol/l (244 to 4880 μg/ml). Rojas et al. (1993) demonstrated that intraperitoneal administration of nicotinamide (500 mg/kg) resulted in plasma concentrations of 3.67 mmol/l (448 μg/ml). As nicotinamide is well absorbed, it is expected that its plasma concentrations in mice and rats after per os administration of doses of 500 or 1000 mg/kg are within the range of concentrations that inhibit the production of inflammatory cytokines or PARP-1 activity.

The antinociceptive activity of nicotinamide is in line with the demonstration of its beneficial effects in patients with rheumatoid arthritis. Hoffer (1959) showed that doses varying from 900 to 4000 mg per day improved joint function of the patients by decreasing joint stiffness, edema, deformity and pain. The doses (500 and 1000 mg/kg) of nicotinamide that induced antinociceptive and anti-inflammatory effects in mice and rats fit with those used in human studies when the formula for dose translation based on body surface area is applied (Reagan-Shaw et al., 2008).

Although our results clearly demonstrate marked antinociceptive and anti-inflammatory effects induced by nicotinamide, a limitation of the study refers to the administration of the compound before the injection of the inflammatory stimuli. In a typical clinical use, anti-inflammatory drugs are administered after pain and inflammation is present. It has been shown that anti-inflammatory drugs more efficiently prevent than reverse the inflammatory edema in experimental models (Zhang et al., 1997). Thus, to fully appreciate the potential of nicotinamide as an anti-inflammatory, it should be administered after the induction of the inflammatory response. However, the beneficial effects induced by nicotinamide in patients with established rheumatoid arthritis (Hoffer, 1959) provide support to the potential anti-inflammatory activity of this compound.

As it has been demonstrated that the nicotinamide isomers, picolinamide and isonicotinamide, also inhibit PARP-1 (Itoh et al., 1984; Uchigata et al., 1982), it was not surprising to observe that both also inhibited the second phase of the nociceptive response induced by formalin and the paw edema induced by carrageenan. Similarly to what was observed for nicotinamide, the isomers induced an antinociceptive effect that was not associated with motor incoordination. Although nicotinamide and its isomers inhibit PARP-1, physical–chemical analysis demonstrated that they differ in their steric and electrostatic interactions (Borba et al., 2008; Seliger and Zagar, 2008) which may contribute to different biological activities, such as the lethality induced by higher doses of picolinamide, the isomer that possesses the side chain in the *ortho* position of pyridine ring. Regarding nicotinamide toxicity, it was demonstrated that the LD_{50} for mice is 4.5 g/kg after p.o. administration (Hoffer, 1967). No information is available about toxicity of the nicotinamide isomers, isonicotinamide and picolinamide. Although the isomers of nicotinamide, picolinamide and isonicotinamide, are not precursors of NAD^+, the inhibition of PARP-1 induced by these compounds can contribute to the maintenance of adequate concentrations of this coenzyme. Thus, nicotinamide and its isomers may differ in relation to the mechanisms that contribute to the maintenance of adequate levels of NAD^+, in other words, nicotinamide might act in direct and indirect ways, while the isomers would act indirectly.

Concluding, our results represent the first demonstration of the activity of nicotinamide in different experimental models of nociceptive and inflammatory pain and also provide further support to its anti-inflammatory activity. The nicotinamide isomers, picolinamide and isonicotinamide, also present similar activities. The demonstration of new activities for nicotinamide is important as it may contribute to expand its use in the treatment of other pathological conditions. This is particularly relevant as nicotinamide has already been approved for clinical use and experience to date suggests that the ratio of risk to benefit of its long-term use would be highly favorable.

Acknowledgments

We thank Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) for financial support.

References

Bischoff U, Vogel W, Safronov BV. Na^+-activated K^+ channels in small dorsal root ganglion neurones of rat. J Physiol 1998;510:743–54.
Borba A, Gomez-Zavaglia A, Fausto R. Molecular structure, vibrational spectra, quantum chemical calculations and photochemistry of picolinamide and isonicotinamide isolated in cryogenic inert matrixes and in the neat low-temperature solid phases. J Phys Chem A 2008;112:45–7.
Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 1997;389:816–24.
Caterina MJ, Rosen TA, Tominaga M, Brake AJ, Julius D. A capsaicin-receptor homologue with a high threshold for noxious heat. Nature 1999;398:436–41.
Chen YL, Le Vraux V, Giroud JP, Chauvelot-Moachon L. Anti-tumor necrosis factor properties of non-peptide drugs in acute-phase responses. Eur J Pharmacol 1994;271:319–27.
Crowley CL, Payne CM, Bernstein H, Bernstein C, Roe D. The NAD^+ precursors, nicotinic acid and nicotinamide protect cells against apoptosis induced by a multiple stress inducer, deoxycholate. Cell Death Differ 2000;7:314–26.
Cunha JM, Cunha FQ, Poole S, Ferreira SH. Cytokine-mediated inflammatory hyperalgesia limited by interleukin-1 receptor antagonist. Br J Pharmacol 2000;130:1418–24.
Cuzzocrea S, Costantino G, Zingarelli B, Caputi AP. Protective effects of poly (ADP-ribose) synthase inhibitors in zymosan-activated plasma induced paw edema. Life Sci 1999a;65:957–64.
Cuzzocrea S, Sautebin L, Costantino G, Rombola L, Mazzon E, Caputi AP. Regulation of prostaglandin production by inhibition of poly (ADP-ribose) synthase in carrageenan-induced pleurisy. Life Sci 1999b;65:1297–304.
Cuzzocrea S, Pisano B, Dugo L, Ianaro A, Maffia P, Patel NS, et al. Rosiglitazone, a ligand of the peroxisome proliferator-activated receptor-gamma, reduces acute inflammation. Eur J Pharmacol 2004;483:79–93.
Dai L, Garg V, Sanguinetti MC. Activation of Slo2.1 channels by niflumic acid. J Gen Physiol 2010;135:275–95.
Fischer BD, Miller LL, Henry FE, Picker MJ, Dykstra LA. Increased efficacy of micro-opioid agonist-induced antinociception by metabotropic glutamate receptor antagonists in C57BL/6 mice: comparison with (−)-6-phosphonomethyl-deca-hydroisoquinoline-3-carboxylic acid (LY235959). Psychopharmacology 2008;198:271–8.
Fukuzawa M, Satoh J, Muto G, Muto Y, Nishimura S, Miyaguchi S, et al. Inhibitory effect of nicotinamide on in vitro and in vivo production of tumor necrosis factor-alpha. Immunol Lett 1997;59:7–11.
Gallí M, Van Gool F, Leo O. Sirtuins and inflammation: friends or foes? Biochem Pharmacol 2011;81:569–76.
Gaskill BN, Rohr SA, Pajor EA, Lucas JR, Garner JP. Some like it hot: mouse temperature preferences in laboratory housing. Appl Anim Behav Sci 2009;116:279–85.
Giansanti V, Donà F, Tillhon M, Scovassi AI. PARP inhibitors: new tools to protect from inflammation. Biochem Pharmacol 2010;80:1869–77.
Gordon CJ. Thermal biology of the laboratory rat. Physiol Behav 1990;47:963–91.
Grange PA, Raingeaud J, Calvez V, Dupin N. Nicotinamide inhibits Propionibacterium acnes-induced IL-8 production in keratinocytes through the NF-kB and MAPK pathways. J Dermatol Sci 2009;56:106–12.
Griffin RJ, Curtin NJ, Newell DR, Golding BT, Durkacz BW, Calvert AH. The role of inhibitors of poly(ADP-ribose) polymerase as resistance-modifying agents in cancer therapy. Biochimie 1995;77:408–22.
Handy RL, Moore PK. A comparison of the effects of L-NAME, 7-NI and L-NIL on carrageenan-induced hindpaw oedema and NOS activity. Br J Pharmacol 1998;123:1119–26.
Hassa PO, Hottiger MO. The functional role of poly(ADP-ribose)polymerase 1 as novel coactivator of NF-kappaB in inflammatory disorders. Cell Mol Life Sci 2002;59:1534–53.

Hegyi J, Schwartz RA, Hegyi V. Pellagra: dermatitis, dementia, and diarrhea. Int J Dermatol 2004;43:1–5.
Hoane MR, Akstulewicz SL, Toppen J. Treatment with vitamin B_3 improves functional recovery and reduces GFAP expression following traumatic brain injury in the rat. J Neurotrauma 2003;20:1189–98.
Hoane MH, Tan AA, Pierce JL, Anderson GD, Smith DC. Nicotinamide treatment reduces behavioral impairments and provides cortical protection after fluid percussion injury in the rat. J Neurotrauma 2006a;23:1535–48.
Hoane MR, Gilbert DR, Holland MA, Pierce JL. Nicotinamide reduces acute cortical neuronal death and edema in the traumatically injured brain. Neurosci Lett 2006b;408:35–9.
Hoffer A. Treatment of arthritis by nicotinic acid and nicotinamide. Can Med Assoc J 1959;81:235–8.
Hoffer A. Biochemistry of nicotinic acid and nicotinamide. Psychosomatics 1967;8: 95–100.
Hoorens A, Pipeleers D. Nicotinamide protects human beta cells against chemically-induced necrosis, but not against cytokine-induced apoptosis. Diabetologia 1999;42:55–9.
Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 1987;30:103–14.
Ijichi H, Ichiyama A, Hayaishi O. Studies on the biosynthesis of nicotinamide adenine dinucleotide. 3. Comparative in vivo studies on nicotinic acid, nicotinamide, and quinolinic acid as precursors of nicotinamide adenine dinucleotide. J Biol Chem 1966;241:3701–7.
Itoh H, Okajima F, Ui M. Conversion of adrenergic mechanism from an alpha- to a beta-type during primary culture of rat hepatocytes. Accompanying decreases in the function of the inhibitory guanine nucleotide regulatory component of adenylate cyclase identified as the substrate of islet-activating protein. J Biol Chem 1984;259: 15464–73.
Jara-Oseguera A, Simon SA, Rosenbaum T. TRPV1: on the road to pain relief. Curr Mol Pharmacol 2008;1:255–69.
Junod A, Lambert AE, Stauffacher W, Renold AE. Diabetogenic action of streptozotocin: relationship of dose to metabolic response. J Clin Invest 1969;48:2129–39.
Kao SJ, Liu DD, Su CF, Chen HI. Niacinamide abrogates the organ dysfunction and acute lung injury caused by endotoxin. J Cardiovasc Pharmacol 2007;50:333–42.
Kort ME, Atkinson RN, Thomas JB, Drizin I, Johnson MS, Secrest MA, et al. Subtype-selective Na(v)1.8 sodium channel blockers: identification of potent, orally active nicotinamide derivatives. Bioorg Med Chem Lett 2010;20:6812–5.
Lapin IP. Nicotinamide, inosine and hypoxanthine, putative endogenous ligands of the benzodiazepine receptor, opposite to diazepam are much more effective against kynurenine-induced seizures than against pentylenetetrazol-induced seizures. Pharmacol Biochem Behav 1981;14:589–93.
Lim SK, Kim JC, Moon CJ, Kim GY, Han HJ, Park SH. Formaldehyde induces apoptosis through decreased Prx 2 via p38 MAPK in lung epithelial cells. Toxicology 2010;271:100–6.
Mathie A. Ion channels as novel therapeutic targets in the treatment of pain. J Pharm Pharmacol 2010;62:1089–95.
McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M, et al. TRPA1 mediates formalin-induced pain. Proc Natl Acad Sci U S A 2007;104: 13525–30.
Miesel R, Kurpisz M, Kröger H. Modulation of inflammatory arthritis by inhibition of poly(ADP-ribose) polymerase. Inflammation 1995;19:379–87.
Nakamura A, Fujita M, Shiomi H. Involvement of endogenous nitric oxide in the mechanism of bradykinin-induced peripheral hyperalgesia. Br J Pharmacol 1996;117:407–12.
Nuwer MO, Picchione KE, Bhattacharjee A. PKA-induced internalization of slack KNa channels produces dorsal root ganglion neuron hyperexcitability. J Neurosci 2010;30:14165–72.
Oliver FJ, Menissier-De Murcia J, Nacci C, Decker P, Andriantsitohaina R, Muller S, et al. Resistance to endotoxic shock as a consequence of defective NF-kappaB activation in poly(ADP-ribose) polymerase-1 deficient mice. EMBO J 1999;18:4446–54.
Pero RW, Axelsson B, Siemann D, Chaplin D, Dougherty G. Newly discovered anti-inflammatory properties of the benzamides and nicotinamides. Mol Cell Biochem 1999;193:119–25.
Petrack B, Greengard P, Kalinsky H. On the relative efficacy of nicotinamide and nicotinic acid as precursors of nicotinamide adenine dinucleotide. J Biol Chem 1966;241:2367–72.
Plone MA, Emerich DF, Lindner MD. Individual differences in the hotplate test and effects of habituation on sensitivity to morphine. Pain 1996;66:265–70.
Radons J, Heller B, Burkle A, Hartmann B, Rodriguez ML, Kroncke KD, et al. Nitric oxide toxicity in islet cells involves poly(ADP-ribose)polymerase activation and concomitant NAD^+ depletion. Biochem Biophys Res Commun 1994;199:1270–7.
Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J 2008;22:659–61.
Rojas A, Hodgkiss RJ, Stratford MR, Dennis MF, Johns H. Pharmacokinetics of varying doses of nicotinamide and tumour radiosensitisation with carbogen and nicotinamide: clinical considerations. Br J Cancer 1993;68:1115–21.
Salvemini D, Wang ZQ, Wyatt PS, Bourdon DM, Marino MH, Manning PT, et al. Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. Br J Pharmacol 1996;118:829–38.
Sauve AA, Moir RD, Schramm VL, Willis IM. Chemical activation of Sir2-dependent silencing by relief of nicotinamide inhibition. Mol Cell 2005;17:595–601.
Seliger J, Zagar V. 14N NQR study of nicotinamide and related compounds. Magn Reson Chem 2008;46:58–62.
Souza ALS, Moreira FA, Almeida KR, Bertollo CM, Costa KA, Coelho MM. In vivo evidence for a role of protein kinase C in peripheral nociceptive processing. Br J Pharmacol 2002;135:239–47.
Stevens MJ, Li F, Drel VR, Abatan OI, Kim H, Burnett D, et al. Nicotinamide reverses neurological and neurovascular deficits in streptozotocin diabetic rats. J Pharmacol Exp Ther 2007;320:458–64.
Szabo C. Role of poly(ADP-ribose)synthetase in inflammation. Eur J Pharmacol 1998;350:1–19.
Tamsett TJ, Picchione KE, Bhattacharjee A. NAD+ activates KNa channels in dorsal root ganglion neurons. J Neurosci 2009;29:5127–34.
Tian LJ, Du YR, Xiao Y, Lv ZM, Yu YQ, Cui XY, et al. Mediating roles of the vanilloid receptor TRPV1 in activation of rat primary afferent nociceptive neurons by formaldehyde. Sheng Li Xue Bao 2009;61:404–16.
Tjolsen A, Berge O-G, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain 1992;51:5–17.
Tonussi CR, Ferreira SH. Mechanism of diclofenac analgesia: direct blockade of inflammatory sensitization. Eur J Pharmacol 1994;251:173–9.
Uchigata Y, Yamamoto H, Kawamura A, Okamoto H. Protection by superoxide dismutase, catalase, and poly(ADP-ribose) synthetase inhibitors against alloxan- and streptozotocin-induced islet DNA strand breaks and against the inhibition of proinsulin synthesis. J Biol Chem 1982;257:6084–8.
Ungerstedt JS, Blomback M, Soderstrom T. Nicotinamide is a potent inhibitor of proinflammatory cytokines. Clin Exp Immunol 2003;131:48–52.
Villarreal CF, Sachs D, Funez MI, Parada CA, de Queiroz Cunha F, Ferreira SH. The peripheral pro-nociceptive state induced by repetitive inflammatory stimuli involves continuous activation of protein kinase A and protein kinase C epsilon and its Na(V)1.8 sodium channel functional regulation in the primary sensory neuron. Biochem Pharmacol 2009;77:867–77.
Vinegar R, Truax JF, Selph JL, Johnston PR, Venable AL, McKenzie KK. Pathway to carrageenan-induced inflammation in the hind limb of the rat. Fed Proc 1987;46: 118–26.
Virág L, Szabo C. The therapeutic potential of poly(ADP-ribose) polymerase inhibitors. Pharmacol Rev 2002;54:375–429.
Westaway SM, Thompson M, Rami HK, Stemp G, Trouw LS, Mitchell DJ, et al. Design and synthesis of 6-phenylnicotinamide derivatives as antagonists of TRPV1. Bioorg Med Chem Lett 2008;18:5609–13.
Zhang Y, Shaffer A, Portanova J, Seibert K, Isakson PC. Inhibition of cyclooxygenase-2 rapidly reverses inflammatory hyperalgesia and prostaglandin E2 production. J Pharmacol Exp Ther 1997;283:1069–75.
Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–10.