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Opioid pathways activation mediates the activity of nicorandil in experimental models of nociceptive and inflammatory pain

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ABSTRACT

We have previously demonstrated that nicorandil inhibits the second phase of the nociceptive response induced by formaldehyde. In the present study, we evaluated the effects induced by nicorandil in other models of nociceptive and inflammatory pain in mice and also whether opioid pathways activation mediates its activity. As we have previously demonstrated, per os (p.o.) administration of nicorandil (50, 100 or 150 mg/kg; −1 h) inhibited the second phase of the nociceptive response induced by intraplantar (i.pl.) injection of formaldehyde. Nicorandil (50, 100 or 150 mg/kg; p.o., −1 h) also exhibited activity in models of inflammatory pain induced by i.pl. injection of carrageenan (300 μg) and nociceptive pain induced by exposure to noxious heat (50 °C). Intraperitoneal (i.p.) administration of the opioid antagonist naltrexone (1, 5 or 10 mg/kg, −30 min) attenuated or abolished the antinociceptive activity of nicorandil (100 mg/kg, p.o.) in the three experimental pain models. In conclusion, we demonstrate that nicorandil exhibits activity in different models of nociceptive and inflammatory pain. The demonstration that the antinociceptive effect induced by nicorandil is markedly attenuated by an opioid antagonist provides solid information about an important mechanism mediating the activity of this antianginal drug. Altogether, our data suggest that the clinical pain relief induced by nicorandil in heart ischemic conditions may result from both vasodilation and intrinsic analgesic activity.

1. Introduction

Nicorandil (2-nicotinamide ethyl nitrate; Fig. 1), a vasodilator drug characterized by the coupling of a nitric oxide (NO) donor to nicotinamide, has been approved for the treatment of patients with angina pectoris (El-Moselby et al., 2009; Frampton et al., 1992). It has been demonstrated that nicorandil releases NO, activates guanylyl cyclase and opens ATP-dependent potassium channels (Barbato, 2005; Kukovetz et al., 1992; Yasuda et al., 2001). NO donors may relieve the pain associated with angina due to their vasodilator property, thus reducing the accumulation of metabolites resulting from myocardial ischemia (Abrams, 1985; Fung, 1993). However, it has been acknowledged that NO donors may also exhibit an intrinsic analgesic activity that is not associated with their ability to promote vasodilation. Many NO donors, including some that have been approved for clinical use, exhibit activity in experimental models of pain after peripheral (Duarte et al., 1990; Soares et al., 2000) or central (Sousa and Prado, 2001) administration.

The first evidence that nicorandil may inhibit the nociceptive processing was provided by showing that its central administration, while not inducing antinociception per se, enhances the effect induced by morphine, fentanyl, bethanechol and clonidine in experimental models of nociceptive pain induced by heat in rats (Asano et al., 2000; Yamazumi et al., 2001). Recently, we demonstrated that systemic administration of nicorandil induces antinociceptive effect in the formalin test, an experimental model of pain that exhibits both a nociceptive and an inflammatory profile (Dutra et al., 2013). We have also shown that this activity is

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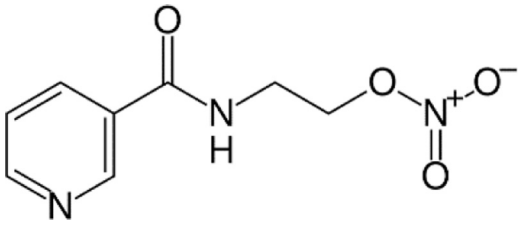

Fig. 1. Chemical structure of nicorandil.

partially mediated by activation of guanylyl cyclase, but not ATP-dependent potassium channels (Dutra et al., 2013).

The involvement of guanylyl cyclase activation in the antinociceptive activity of nicorandil provides the rationale for the present study. First, it has been demonstrated that NO may stimulate the release of endogenous opioids (Armstead, 1998; Ohgami et al., 2010; Zelinski et al., 2009). Second, agonists of different opioid receptors, including morphine, synthetic drugs and endogenous opioids, increases cGMP levels in different tissues (Askew and Charalampous, 1976; Bhargava and Cao, 1997; Fóris et al., 1986; Muraki et al., 1984). Finally, it has been shown that the antinociceptive activity of some opioid drugs is inhibited by guanylyl cyclase inhibitors (Amarante and Duarte, 2002; Ferreira et al., 1991; Granados-Soto et al., 1997).

Thus, in the present study, we extended the investigation on the antinociceptive activity of nicorandil by evaluating its effects in other models of pain and also whether activation of opioid pathways could mediate such activity.

2. Material and methods

2.1. Animals

Male Swiss mice (25–30 g) were used throughout the study and had free access to food and water. The animals were kept in a room with 12 h light–dark cycle and temperature of 27 °C, which corresponds to the thermoneutral zone for rodents, for at least 3 days before the experiment to allow acclimatization. This study was approved by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais (Protocol 131/11) and the experiments were conducted according to the ethical guidelines for investigation of experimental pain in conscious animals (Zimmermann, 1986).

2.2. Drugs

Nicorandil (purity > 99.0%) was synthesized at the Department of Chemistry, Federal University of Minas Gerais. Dipyrone, naltrexone, λ-carrageenan and formaldehyde solution were purchased from Sigma, USA. The suspension of nicorandil was prepared in carboxymethylcellulose (CMC; 0.5% w/v in sterile saline). Dipyrone, naltrexone and formaldehyde solutions and carrageenan suspension were prepared in sterile saline.

2.3. Evaluation of the nociceptive response induced by formaldehyde

On the experiment day, the animals were placed under glass funnels (18 cm diameter and 14 cm high) about 30 min before injection of formaldehyde to allow acclimatization. Formaldehyde (1.84%, 10 μl) was injected via intraplantar (i.pl.) route in the right hindpaw of the animals. Nicorandil (50, 100 or 150 mg/kg) or vehicle (CMC 0.5%, 10 ml/kg) were administered per os (p.o.) 1 h before the injection of formaldehyde. In the present study, we selected higher doses of nicorandil as in our previous study (Dutra et al., 2013) doses lower than 50 mg/kg did not induce a statistically significant reduction of the nociceptive response. Immediately after the injection, each mouse was placed again under the glass funnel. The amount of time that the animals licked the injected paw was measured between 0–5 min (first phase) and 15–30 min (second phase) after the injection of formaldehyde (Tjølsen et al., 1992). To evaluate mechanisms mediating the antinociceptive activity of nicorandil, naltrexone (1, 5 or 10 mg/kg) or vehicle (sterile saline, 4 ml/kg) were administered via the intraperitoneal (i.p.) route 30 min before nicorandil (100 mg/kg, p.o.).

2.4. Evaluation of the mechanical allodynia induced by carrageenan

Mechanical allodynia was measured by using an electronic von Frey apparatus (Model EFF 301, Insight, Brazil). After acclimatization of the animals to the experimental apparatus (1 h/day during 3 days), the basal paw withdrawal threshold of each mouse was determined (mean of three measurements). Then, the animals were divided into the experimental groups in such a way that the mean withdrawal thresholds of the different groups were similar. On the experiment day, carrageenan (300 μg, 20 μl) was injected via the intraplantar (i.pl.) route 1 h after p.o. administration of nicorandil (50, 100 or 150 mg/kg) or vehicle (CMC 0.5%, 10 ml/kg). The paw withdrawal threshold of each animal was again measured at 2, 4 and 6 h after carrageenan injection. The withdrawal thresholds were expressed in grams. To evaluate mechanisms mediating the antinociceptive activity of nicorandil, naltrexone (1, 5 or 10 mg/kg) or vehicle (sterile saline, 4 ml/kg) were administered i.p. 30 min before nicorandil (100 mg/kg, p.o.).

2.5. Evaluation of the nociceptive response induced by noxious heat

A hot plate apparatus (Model EFF 361, Insight, Brazil) was used to evaluate the nociceptive response induced by heat. Based on previous experiments, a temperature of 50 °C was selected. One hour after p.o. administration of nicorandil (50 or 100 mg/kg), dipyrone (500 mg/kg, positive control) or vehicle (CMC 0.5%, 10 ml/kg), each animal was placed on the hot plate. The latency (s) 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 time was 50 s to avoid tissue damage. To evaluate mechanisms mediating the antinociceptive activity of nicorandil, naltrexone (5 or 10 mg/kg) or vehicle (sterile saline, 4 ml/kg) were administered i.p. 30 min before nicorandil (100 mg/kg, p.o.).

2.6. Statistical analysis

Data were presented as mean ± standard error of the mean. One-way ANOVA, followed by Newman–Keuls post hoc test was used to analyze the data. A P value < 0.05 was considered significant and statistical analysis was conducted using GraphPrism 5.0 for Windows.

3. Results

Intraplantar injection of formaldehyde induced the characteristic biphasic nociceptive response. Confirming previous results (Dutra et al., 2013), nicorandil (50, 100 or 150 mg/kg), administered 1 h before formaldehyde injection, inhibited the second, but not the first phase of the nociceptive response (Fig. 2A). Next, we investigated whether such activity could be the result of the activation of endogenous opioid pathways. We observed that the antinociceptive activity of nicorandil (100 mg/kg) was abolished