European Journal of Pharmacology 737 (2014) 41–46

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Neuropharmacology and analgesia

The role of gaseous neurotransmitters in the antinociceptive effects of morphine during acute thermal pain

Gemma Gou, Sergi Leánez, Olga Pol *

Grup de Neurofarmacologia Molecular, Institut d'Investigació Biomèdica Sant Pau & Institut de Neurociències, Universitat Autònoma de Barcelona, Facultat de Medicina. Edifici M2-115, Barcelona 08193, Spain

ARTICLE INFO

Article history:
Received 20 December 2013
Received in revised form
29 April 2014
Accepted 7 May 2014
Available online 15 May 2014

Keywords:
Carbon monoxide
Nitric oxide
Morphine
Antinociception
Thermal nociception
Pain

ABSTRACT

Treatment with a carbon monoxide-releasing molecule (tricarbonyldichlororuthenium(II) dimer, CORM-2) or a classical inducible heme oxygenase (HO-1) inducer (cobalt protoporphyrin IX, CoPP) enhanced the anti-nociceptive effects of morphine during chronic pain but the role played by these compounds in acute thermal nociception was not evaluated. The effects of CORM-2 and CoPP treatments on the local antinociceptive actions of morphine and their interactions with nitric oxide during acute pain were evaluated by using wild type (WT), neuronal (nNOS-KO) or inducible (iNOS-KO) nitric oxide synthase knockout mice and assessing their thermal nociception to a hot stimulus with the hot plate test. Our results showed that the absence of nNOS or iNOS genes did not alter licking and jumping responses nor the antinociceptive effects produced by morphine indicating that the local thermal inhibitory effects produced by this drug in the absence of inflammation or injury are not mediated by the nitric oxide pathway triggered by nNOS or iNOS enzymes. Moreover, while the systemic administration of CORM-2 or CoPP inhibited licking and jumping latencies in all genotypes, these treatments only enhanced the local inhibition of jumping latencies produced by morphine in WT and nNOS-KO mice which effects were reversed by the peripheral administration of an HO-1 inhibitor. These data indicate that the co-administration of morphine with CORM-2 or CoPP produced remarkable local antinociceptive effects in WT and nNOS-KO mice and reveal that a significant interaction between carbon monoxide and nitric oxide systems occurs on the local antinociceptive effects produced by morphine during acute thermal nociception.

1. Introduction

Nitric oxide synthesized either by neuronal (nNOS) or inducible (iNOS) nitric oxide synthase mediates numerous chronic pain symptoms via cGMP-PKG pathway activation (LaBuda et al., 2006; Schmidtko et al., 2008). Accordingly, the expression of both enzymes is up-regulated in the spinal cord and dorsal root ganglia from animals with chronic pain (De Alba et al., 2006; Hervera et al., 2010b). Moreover, the hypersensitivity induced by nerve injury was significantly diminished in nNOS (nNOS-KO) and iNOS (iNOS-KO) knockout animals (Kuboyama et al., 2011; Hervera et al., 2012) or reversed by the administration of selective nNOS, iNOS, guanylate cyclase or PKG inhibitors (De Alba et al., 2006; Schmidtko et al., 2008; Hervera et al., 2010a). However, the role played by nitric oxide during acute thermal nociception has not been completely evaluated.

Carbon monoxide, another gaseous neurotransmitter, synthetized by inducible (HO-1) and constitutive heme oxygenase enzymes, also regulates nociception by the activation of the cGMP-PKG signalling pathway. However, while the over-expression of the constitutive heme oxygenase exerts a pronociceptive effect after nerve injury (Li and Clark, 2003; Hervera et al., 2012), the increased expression of HO-1 exerts potent anti-inflammatory and antinociceptive effects during inflammatory and neuropathic pain (Fan et al., 2011; Hervera et al., 2012; Negrete et al., 2014). Indeed the administration of an HO-1 inducer compound, such as cobalt protoporphyrin IX (CoPP) or a carbon monoxide-releasing molecule, such as tricarbonyldichlororuthenium(II)dimer (CORM-2), a new class of chemical agents able to reproduce several biological effects of HO-1-derived carbon monoxide (Motterlini et al., 2002), inhibits chronic pain (Guillén et al., 2008; Hervera et al., 2012). But the exact contribution of carbon monoxide synthesized by HO-1, in the modulation of thermal nociception as well as their possible interaction with the nitric oxide system has not been investigated.

It is well known that the local administration of morphine elicits antinociceptive effects during inflammatory and neuropathic pain which effects are produced by the activation of the peripheral nitric oxide-cGMP-PKG-ATP-sensitive K+ channels signalling pathway (Leánez et al., 2009; Cunha et al., 2010; Hervera et al., 2011). Recent

* Corresponding author. Tel.: +34 619 757 054; fax: +34 935 811 573.
E-mail address: opol@santpau.es (O. Pol).

http://dx.doi.org/10.1016/j.ejphar.2014.05.004
0014-2999/

studies also demonstrate that the administration of CORM-2 and CoPP enhances the local antiallodynic and antihyperalgesic effects produced by morphine during neuropathic pain (Hervera et al., 2013a), but the possible involvement of carbon monoxide synthetized by HO-1 in the thermal antinociceptive effects produced by morphine is still unknown.

Therefore in wild type (WT), nNOS-KO and iNOS-KO mice, the local thermal antinociceptive effects produced by morphine administered alone or combined with the intraperitoneal administration of CORM-2 and CoPP are evaluated. Moreover, the thermal antinociceptive effects of morphine combined with the subplantar administration of an HO-1 inhibitor, tin protoporphyrin IX (SnPP), are also assessed.

2. Material and methods

2.1. Animals

Experiments were performed in male nNOS-KO and iNOS-KO mice (C57BL/6J background) purchased from Jackson Laboratories (Bar Harbor, ME, USA) as well as in WT mice with the same genetic background (C57BL/6J) acquired from Harlan Laboratories (Barcelona, Spain). All mice weighing 21–25 g were housed under 12 h/12 h light/dark conditions in a room with controlled temperature (22 °C) and humidity (66%). Animals had free access to food and water and were used after a minimum of 6 days acclimatization to the housing conditions. All experiments were conducted between 9:00 a.m. and 5:00 p.m.. All experiments were carried out according to the Ethical Guidelines of the International Association for the Study of Pain and approved by the local ethical committee of our Institution (Comissió d'Ètica en l'Experimentació Animal i Humana de la Universitat Autònoma de Barcelona)

2.2. Induction of acute pain

Thermal nociception to a hot stimulus was assessed by using the hot/cold-plate analgesia meter (Ugo Basile, Italy). Briefly, mice were placed into a Plexiglas cylinder (diameter, 20 cm; height, 18 cm) on a metal surface maintained at 52.5 °C. The time between placement and licking of the hindpaws and jumping was recorded. To avoid tissue damage of hindpaws, the cut-off was 60 and 240 s, respectively. Only one test per animal was performed because repeated measures might cause profound latency changes (Mogil et al., 1999).

2.3. Experimental protocol

In a first set of experiments, we evaluated the baseline response to an acute thermal stimulus in WT, nNOS-KO and iNOS-KO mice. In a second set of experiments, we investigated the inhibitory effects produced by the subplantar administration of morphine in WT, nNOS-KO and iNOS-KO mice. In a third set of experiments, the effects produced by the intraperitoneal administration of 2.5 mg/kg of CORM-2 or 1 mg/kg of CoPP alone or combined with a low dose of morphine (10 μg) or vehicle subplantarly administered were also evaluated ($n=8$ animals per group). In the last set of experiments, we evaluated the effects produced by the subplantar administration of 290 μg of SnPP alone or combined with the subplantar administration of a high dose of morphine (100 μg) ($n=8$ animals per group).

The doses of CORM-2, CoPP and SnPP were selected from our preliminary experiments and in accordance to other studies (Nascimento and Branco, 2007, 2009; Rosa et al., 2008; Hervera et al., 2012, 2013b; Negrete et al., 2014). The doses of morphine were selected according as the ones that produced a minimal thermal antinociceptive effect in each genotype.

2.4. Drugs

CORM-2 was purchased from Sigma-Aldrich (St. Louis, MO), CoPP and SnPP from Frontier scientific (Livchem GmbH & Co, Frankfurt, Germany). Morphine hydrochloride was obtained from Alcaiber S.A. (Madrid, Spain). CORM-2, CoPP and SnPP were dissolved in dimethyl sulfoxide (DMSO; 1% solution in saline). Morphine was dissolved in saline solution (0.9% NaCl). All drugs were freshly prepared before use. CORM-2 and CoPP were intraperitoneally administered 3 h before testing, in a final volume of 10 ml/kg. Morphine and SnPP were subplantarly administered 30 min before behavioral testing in a final volume of 20 μl. For each group treated with a drug the respective control group received the same volume of vehicle.

2.5. Statistical analysis

Data are expressed as mean ± standard error of the mean (S.E.M.). The comparison of the basal nociceptive responses between genotypes was evaluated by using a one way ANOVA followed by the Student Newman Keuls test. For each knockout mice and behavioral evaluated the effects produced by the administration of different doses of morphine were also evaluated by using a one way ANOVA followed by the Student Newman Keuls test. The comparison of the effects produced by a dose of morphine on the inhibition of licking or jumping latencies between genotypes was also performed by using a one way ANOVA followed by the Student Newman Keuls test. The evaluation of the effects produced by morphine alone or combined with CORM-2, CoPP or SnPP for each genotype and behavioral evaluated was also assessed by using a one way ANOVA followed by the Student Newman Keuls test.

Antinociception is expressed as the percentage of maximal possible effect, where the test latencies pre-(baseline) and post-drug administration are compared and calculated according to the following equations, respectively:

$$\text{Maximal possible effect (\%)} = [(\text{drug} - \text{baseline})/(\text{cut-off} - \text{baseline})] \times 100$$

A value of $P < 0.05$ was considered as a significant.

3. Results

3.1. Thermal nociception in WT, nNOS-KO and iNOS-KO mice

The latencies to display hindpaw licking or jumping in the hot plate were similar in WT, nNOS-KO and iNOS-KO mice as shown in Fig. 1. For each behavioral evaluated, the one way ANOVA did not reveal significant differences between genotypes.

3.2. The local antinociceptive effects of morphine in WT, nNOS-KO and iNOS-KO mice

The antinociceptive effects produced by the local administration of different doses of morphine in WT, nNOS-KO and iNOS-KO mice have been evaluated. Our results show that the subplantar administration of different doses (10–100 μg) of morphine inhibited the licking (Fig. 2A) and jumping (Fig. 2B) latencies in a dose dependent manner in WT, nNOS-KO and iNOS-KO mice.

In all genotypes, the one way ANOVA showed a significant effect produced by 25, 50 and 100 μg of morphine on the inhibition of licking and jumping latencies ($P < 0.001$), as compared to vehicle treated mice (one way ANOVA, followed by the Student Newman Keuls test). Our results also indicate that non-significant differences could be observed between genotypes when compared with the effects produced by different doses of morphine on the inhibition of licking or jumping latencies.