Neuroscience Letters 633 (2016) 77–81

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

Melatonin as a potential counter-effect of hyperalgesia induced by neonatal morphine exposure

Joanna Ripoll Rozisky[a,b,d], Vanessa Leal Scarabelot[a,c,d], Carla de Oliveira[a,b,d], Isabel Cristina de Macedo[a,c,d], Alícia Deitos[a,b,d], Gabriela Laste[a,b,d], Wolnei Caumo[a,b], Iraci L.S. Torres[a,b,c,*]

[a] Pain Pharmacology and Neuromodulation Laboratory, Preclinical Research, Department of Pharmacology, Institute of Basic Health Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, 90050-170, Brazil
[b] Postgraduate Program in Medical Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, 90035-003, Brazil
[c] Postgraduate Program in Biological Sciences: Physiology, Institute of Health Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, 90050-170, Brazil
[d] Animal Experimentation Unit, Graduate Research Group, Hospital de Clínicas de Porto Alegre, RS, 90035-003, Brazil

HIGHLIGHTS

- Melatonin reduce hyperalgesia induced by neonatal morphine exposure.
- Neonatal morphine exposure promotes long-term changes on nociceptive pathways.
- Repeated low-dose of neonatal morphine induces hyperalgesia in adult rats.
- Melatonin exerts its antinociceptive action by β-endorphin release in the SNC.

ARTICLE INFO

Article history:
Received 20 May 2016
Received in revised form 11 August 2016
Accepted 15 August 2016
Available online 18 August 2016

Keywords:
Formalin test
Hyperalgesia
Melatonin
Morphine
Neonatal rats

ABSTRACT

Morphine administration in the neonatal period can induce long-term effects in pain circuitry leading to hyperalgesia induced by the opioid in adult life. This study explored a new pharmacological approach for reversing this effect of morphine. We focused on melatonin owing its well-known antinociceptive and anti-inflammatory effects, and its ability to interact with the opioid system. We used the formalin test to assess the medium and long-term effects of melatonin administration on hyperalgesia induced by morphine in early life. Newborn rats were divided into two groups: the control group, which received saline, and the morphine group, which received morphine (5 μg subcutaneously [s.c.]) in the mid-scapular area, once daily for 7 days, from P8 (postnatal day 8) until P14. At postnatal days 30 (P30) and 60 (P60), both groups were divided in two subgroups, which received melatonin or melatonin vehicle 30 min before the formalin test. The nociceptive responses were assessed by analyzing the total time spent biting, flicking, and licking the formalin-injected hind paw; these responses were recorded during the first 5 min (neurogenic/acute phase) and from 15 to 30 min (inflammatory/tonic phase). Initially, animals in the morphine/vehicle group showed increased nociceptive behavior in phase II (inflammatory) of the formalin test at P30, and in the neurogenic and inflammatory phases at P60. These increased nociceptive responses were fully reversed by melatonin administration at either age. These findings show that melatonin administration is a potential means for countering hyperalgesia induced by neonatal morphine exposure in young and adult rats.

1. Introduction

Opioid use has increased in the Neonatal Intensive Care Unit over the last few decades, as a consequence of changes and advances in the understanding, identification, and treatment of pain in children [1,2]. In the intensive care unit, opioids offer the

* Corresponding author at: Departamento de Farmacologia–ICBS, UFRGS, Rua Sarmento Leite 500, Sala 305, 90050–170, Porto Alegre, RS, Brazil,
E-mail address: iltorres@hcpa.edu.br (I.L.S. Torres).

http://dx.doi.org/10.1016/j.neulet.2016.08.027
0304-3940/

best means of sedation for mechanical ventilation, and provide analgesia after surgery [3,4].

Despite their widespread use, there are only few studies reporting the long-term effects of opioid exposure during the neonatal period. Our group has shown that neonatal morphine exposure promotes long-term changes in nociceptive pathways, altered glutamate signaling, and lack of an opioid response in adult life [5–7]. In a previous study, we have shown that repeated low-dose morphine administration in neonatal rats induces hyperalgesia in adult life, which could be reversed by a glutamate receptor antagonist [7]. In addition, in another study, we showed that rats subjected to repeated neonatal morphine administration presented an increased nociceptive response in the formalin test in the medium- and long-term [5,6].

In parallel with these studies, we also investigated the antinociceptive effects of melatonin in animal models of chronic inflammation [8,9]. In the last decade, melatonin, a neurohormone that restores the circadian rhythm [10], has been widely studied owing to its analgesic and anti-inflammatory effects [11,12]. These effects have been demonstrated in human clinical studies [13–16] and animal models [8,17–19]. In a clinical trial, we have shown that the effects of melatonin on pain were independent of improved sleep quality, suggesting a direct effect on pain pathways or on chemicals that regulate pain [15]. Furthermore, other observations have pointed to significant interactions between melatonin and the opioid system [18,20]. Studies have demonstrated that subcutaneous administration of the nonselective opioid receptor antagonist naltrexone partially diminished the antinociceptive effects induced by the oral administration of melatonin [11,20]. These results corroborate previous data that showed, in nondiabetic rats, that melatonin induced antinociceptive, antihyperalgesic [8,21], and anti-allodynic effects [11,20], which could be blocked by a non-selective opioid receptor antagonist.

Thus, considering the antinociceptive effect of melatonin and its relationship with the opioid system, the aim of this study was to verify whether melatonin administration was able to reduce the hyperalgesia promoted by repeated morphine administration in early life.

2. Materials and methods

2.1. Animals

All experiments were performed in accordance with Brazilian Law No. 11.794 of October 8, 2008, and the Guide for the Care and Use of Laboratory Animals 8th edition (2011). Animal handling and all experiments were performed in accordance with international guidelines for animal welfare and measures were taken to minimize animal pain and discomfort and to use only the number of animals necessary to produce reliable scientific data. All experiments and procedures were approved by the Institutional Committee for Animal Care and Use (GPPG-HCPA protocol No. 08-345).

Eight-day-old male Wistar rats were initially divided into two groups: a saline control (C) and the morphine-treated (M) groups. Animals were housed in polypropylene home cages (49 cm × 34 cm × 16 cm) with sawdust-covered floors. All animals were kept on a standard 12-h light/dark cycle (lights on at 07:00 a.m. and lights off at 07:00 p.m.) in a temperature-controlled environment (22 ± 2 °C). Animals had access to water and chow ad libitum. At birth, litters were standardized to contain up to 8 pups per dam, and the pups remained with their mothers until 21 days of age. Rats at P8 were chosen for experiments because it is accepted that animals of this age are at a similar stage of neurological development as that of a human newborn [22]. To control the possible effect of outliers, we excluded rats that did not present any response to behavioral testing.

2.2. Morphine administration

Each animal received saline (control group) or morphine (5 μg subcutaneously [s.c.] in the mid-scapular area) from postnatal day 8 (P8), once a day for 7 days [5,23]. Morphine sulfate (1 ml; Dimorf® at 10 mg/ml, obtained from Hospital de Clínicas de Porto Alegre, Rio Grande do Sul, Brazil) was dissolved in 9 ml of 0.9% saline, and animals were treated at the same time each day (11:00 h). This dose was chosen based on a previous study by Rozisky et al. (2008, 2011, 2012) [5–7]; this was the smallest dose capable of producing analgesia in all animals submitted to the tail-flick test, without inducing classic tolerance to morphine.

2.3. Melatonin administration

The control and morphine groups were subdivided into two groups, each one designed to evaluate the effect of intraperitoneal (i.p.) administration of melatonin 30 min before the formalin test: The first group received 50 mg/kg of melatonin [6,8] (Sigma–Aldrich, São Paulo, Brazil) diluted in 1% ethanol in saline (control/melatonin = CMt, morphine/melatonin = MMt). This dose reversed the hyperalgesia induced by chronic inflammatory pain in a previous experiment conducted by our group [8]. The second group received 1% ethanol in saline (control/vehicle = CV, morphine/vehicle = MV). This dosage was chosen based in our previous experiments in which the melatonin-dose response was evaluated using the formalin test. In this study, we verified that this was the lowest dose able to promote an analgesic effect in the formalin test (data not shown). The number of animals used per group was 5–7.

2.4. Formalin test

The formalin test was performed in 30- and 60-day-old rats, once in each rat, where we observed significant between-group differences in nociceptive behavior [6]. The test was performed as previously described [24–26], with minor modifications. Twenty-four hours before the test, each animal was placed in the chamber for 10 min to familiarize them with the procedure, since the novelty of the apparatus itself could induce antinociception [27].

The animals were injected subcutaneously into the plantar surface of the left hindpaw with 0.17 ml/kg of a 2% formalin solution (Formaldehyde P.A.®; Sigma–Aldrich, São Paulo, Brazil) diluted in 0.9% NaCl (saline). Each animal was observed in a varnished wood cage, measuring 60 × 40 × 50 cm, with the inside lined with glass, and the nociceptive response was recorded for a period of 30 min. This test produces two distinct phases of nociceptive behavior: early, transient phase (phase I; up to 5 min after injection) and a late, persistent phase (phase II; 15–30 min after injection). Phase I is thought to reflect direct stimulation of the primary afferent fibers, predominantly C-fibers (neurogenic pain–acute pain) [28], whereas phase II is dependent on peripheral inflammation (inflammatory pain–tonic pain) [26]. The total duration (seconds) of licking, biting, and flicking of the formalin-injected hind paw was recorded in neurogenic and inflammatory phases. The response to the formalin test was recorded visually/manually. The same observer analyzed all behaviors to avoid measurement biases, and the experimenter was blinded to the treatment (morphine or vehicle; melatonin or vehicle) used during all behavioral recording.

2.5. Statistical analysis

Data were expressed as means ± standard error of the mean (SEM). One-way ANOVA was performed, followed by a multi-