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Research report

Melatonin enhances antinociceptive effects of δ-, but not μ-opioid agonist in mice

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Abstract

This present study examines the effect of melatonin on antinociceptive action induced by opioid agonists in mice using the tail-flick test. When injected either by intraperitoneal (i.p.) (1, 5, 25 mg/kg) or by intracerebroventricular (i.c.v.) (0.25, 0.5, 1 mg/kg) routes, melatonin significantly enhanced the δ-opioid agonist deltorphin I induced antinociception, but not μ-opioid agonist endomorphin-1. Further investigation showed that i.c.v. luzindole (0.5 mg/kg) (an antagonist of melatonin receptor) significantly antagonized the enhanced antinociceptive effect of i.c.v. melatonin. These results demonstrated that melatonin can specifically enhance the antinociception induced by specific opioid receptor agonist (i.e., δ opioid agonist) acting on melatonin receptor and that melatonin may have augmentation effect on analgesia with delta-, but not mu-opioid agonists in mice.

Theme: Sensory systems
Topic: Pain modulation: pharmacology

Keywords: Melatonin; Opioid; Deltorphin I; Endomorphin-1; Antinociception; Mice

1. Introduction

Melatonin is one major hormone secreted by pineal gland. Its receptors are mainly found in the hypothalamus [14]. Melatonin can produce profound analgesic effects in behavioral nociceptive tests in both rats and mice [6,8,24], as well as reduce the pain indexes of patients suffered with cluster headache, without any obvious toxic or side reaction [11]. In other studies, melatonin has been demonstrated to induce analgesia as well as affect opioid antinociception [8,17]. Opioids can produce potent antinociception, but simultaneously also bring many side effects including respiratory depression, nausea, vomiting, constipation, dependence and tolerance, which greatly limit the clinical application of opioids [21]. To reduce these side effects without disturbing the clinical efficacy, opioids are often used combined with other pharmacological agents. It has been reported that some known side effects of morphine, such as dependence and tolerance, could be attenuated when it was co-injected with melatonin [19]. So, melatonin may have potential clinical value as a new and effective adjunctive analgesic.

The interaction between melatonin and opioids has been reported, but the interaction is still under debate. In analgesia, melatonin could be either as an opioid agonist or as an antagonist [5]. For example, antinociceptive response was increased on formalin-induced nociception in mice, when melatonin was given at 20 mg/kg together with diazepam and morphine [17]. And the analgesic effects

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of melatonin would be impaired by the co-treatment of melatonin with opioid antagonist naloxone [8]. But, on the other hand, using the tail flick nociception model, melatonin has been reported to attenuate the morphine-induced analgesia in rats [3]. Co-treatment of melatonin with morphine also reversed the morphine tolerance and dependence in mice [18]. However, the research of its relationship with opioid in analgesia has been only focused on its co-effect with the nonselective agonist and antagonist, morphine and naloxone. There are three major subtypes of opioid receptors: μ, δ and κ. Among these subtypes, μ and δ receptors are mainly related to antinociception, but their interaction with other neurochemicals is different. It has been reported that low doses of naltrexone, a specific opioid antagonist which preferentially binds μ receptors, completely abolished any immuno-augmenting and anti-stress effect of melatonin. In contrast, the δ receptor specific antagonist ICI 174,864 did not exert any effect on the melatonin action [13]. These findings suggest that the different effects might be due to the different interaction mechanisms between melatonin and individual subtype of opioid receptors. However, up to now, the role of melatonin on analgesia induced by different subtypes of opioid receptors has not been studied.

The present study aimed to answer two questions: (a) what is the effect of melatonin on antinociception induced either by μ-opioid receptor agonist or by δ-opioid receptor agonist? and (b) whether this effect can be blocked by the melatonin receptor antagonist luzindole? To find the solutions for these two questions, melatonin was administered by different doses and delivery routes of injection to reveal the drug's antinoceptive effects. With the tail-flick test of mice as the acute nociception model, the interaction between melatonin and endomorphin-1 (high affinity and selectivity μ-opioid receptor agonist [26]) or deltorphin I (high affinity and selectivity δ-opioid receptor agonist [7]) was tested separately, so as to determine the effect of melatonin on analgesia induced by different subtypes of opioid receptors.

2. Materials and methods

2.1. Animals

Male Kunming mice (20.0 ± 1.0 g) were supplied by the Animal Center of the Lanzhou Medical College. They were maintained at room temperature (22 ± 1 °C), with a 12 h light/dark cycle. Food and water were offered ad libitum. Every mouse was used only once. They were sacrificed shortly after the behavioral testing to minimize suffering of the animal. All the protocols in this study were approved by the Ethics Committee of Lanzhou Medical College, China.

2.2. Drugs

The peptides used in this study, deltorphin I (Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH2) and endomorphin-1(Tyr-Pro-Trp-Phe-NH2), were synthesized by the solid-phase peptide synthesis method and purified by high-performance liquid chromatography (HPLC) in our laboratory. Melatonin and luzindole were purchased from Sigma Chemical USA. Melatonin stocks of 100 mg/ml in 100% ethanol were prepared fresh on the days of experiment and diluted with 0.9% NaCl to the appropriate concentrations for injection. The concentration of ethanol was adjusted to 5% per injection for all drug administrations.

2.3. Tail-flick assay

The pain threshold of mice was assessed with warm-water tail-flick test at 49 °C and was measured during 13:00–16:00 h. The latency to the first sign of a rapid tail flick was taken as the behavioral end point. Every mouse was first tested for baseline latency by immersing its tail in the water and recording the response time. Only those mice with the baseline latency within the range of 2–4 s were selected for further studies. To prevent tissue damage, we established a 15 s cut-off time. Following drug administration, tail flick latency was measured every 10 min for 90 min. The i.c.v. administration was performed following the method described by Haley and McCormick [10]. Under light ether anesthesia, an incision was made in the scalp. Based on our pilot experiment by administration and localization of methylene blue dye, the injection site was 1.5 mm lateral from the bregma and 3.0 mm from the surface of the skull. Each drug or the mixture of combination of drugs was administered in one i.c.v injection with a total volume of 5 μl per mouse at a constant rate of 10 μl/min. Drugs were i.p. administered in a volume of 100 μl at a constant rate of 200 μl/min. To investigate the effect of i.p. melatonin on antinociception induced by deltorphin I or endomorphin-1, melatonin was injected by i.p. route, then 10 min later, endomorphin-1 or deltorphin I was i.c.v. administered. Control was pretreated with i.p. 5% ethanol/saline. Cocktails containing different concentrations of melatonin (0.25, 0.5, 1 mg/kg) and deltorphin I (5, 50 nmol/kg) or endomorphin-1 (5, 50 nmol/kg) were used to investigate effect of i.c.v. melatonin on antinociception induced by the i.c.v. administration of the used peptides. The effect of luzindole on the analgesic action of melatonin and deltorphin I was determined by i.c.v. co-injected the cocktails containing melatonin (1 mg/kg), luzindole (0.5 mg/kg) and deltorphin I (50 nmol/kg).

2.4. Statistics

The antinociceptive effect in the above test is calculated as percentage change of tail flick latency from the baseline level according to the formula: percentage change =