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Opioid system mediated anti-nociceptive effect of agomelatine in mice

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ABSTRACT

Aims: This study was planned to examine the antinociceptive efficacy of agomelatine against acute mechanical, thermal, and chemical nociceptive stimuli, as well as to determine the opioid receptor subtypes mediating these effects.
Main methods: Tail-clip, hot-plate, and acetic acid-induced writhing tests were performed to evaluate anti-nociceptive effect. Besides, possible effect of agomelatine on the motor coordination of animals was assessed with a Rota-rod test.
Key findings: Agomelatine (40 mg/kg and 60 mg/kg) significantly prolonged the reaction time of mice in both the tail-clip and hot-plate tests, suggesting the antinociceptive activity is related to both spinal and supraspinal mechanisms. This drug also reduced the number of writhing behaviors indicating the presence of a peripherally mediated antinociceptive effect. Rota-rod testing displayed no notable effect on the motor activity of the animal supporting the conclusion that the observed antinociceptive effect is specific. The agomelatine-induced antinociceptive activity abrogated following pretreatment with naloxone (a non-selective opioid receptor antagonist, 5.48 mg/kg, i.p.), which suggested the participation of opioid mechanisms to the antinociception. The possible contribution of μ, δ and κ subtypes of opioid receptors to the anti-nociceptive effect were evaluated using naloxonazine (7 mg/kg, s.c.), naltrindole (0.99 mg/kg, i.p.), and nor-binaltorphimine (1.03 mg/kg, i.p.), respectively. Pretreatments using these antagonists abolished the antinociceptive activity of agomelatine in all of the nociceptive test paradigms used, which pointed out that μ, δ, and κ opioid receptors participated to the action of agomelatine on pain.
Significance: These results demonstrated the therapeutic potential of agomelatine in the treatment of pain disorders.

1. Introduction

Agomelatine (S-20098), is a novel pharmacological agent with an agonistic effect on the MT_1 and MT_2 melatonin receptors [1,2] and antagonistic effect on the 5-HT_{2C} serotonin receptor [3,4].

Agomelatine is successfully used in the therapy of several mood disorders, in particular, major depression [5,6]. In a randomized, double-blind study comparing the efficacy of agomelatine with venlafaxine, agomelatine has been reported to treat patients with major depression as effectively as venlafaxine, but it was faster-acting and more effective than venlafaxine for the remedy of sleep complaints in these patients [7]. In another randomized, double-blind study, agomelatine has been suggested to be more effective than fluoxetine for treating severe major depressive disorder and reported to be well tolerated by patients [8]. The antidepressant efficacy of this drug has been suggested to be at least as strong as paroxetine and sertraline [9]. It has been suggested that agomelatine has clinically significant advantages not only in terms of good tolerability profile, but also its low incidence in inducing weight gain and sexual dysfunction [9,10].

Agomelatine is also known to be effective in treating a variety of sleep disorders. In addition to the sleep disturbances accompanying affective disorders, agomelatine has been suggested to be useful in sleep disorders frequently seen in patients with Parkinson's disease [11] as well as psychostimulant medication induced insomnia [12] Clinical studies have demonstrated that agomelatine successfully treats various affective disorders such as seasonal affective disorder [13] and generalized anxiety disorder [14].

Preclinical studies in mice, which investigated the efficacy of agomelatine on the central nervous system (CNS), demonstrated that agomelatine has anticonvulsant activity in pentylenetetrazole- or pilocarpine-induced seizures [15,16]. The anticonvulsant activity of the drug has been suggested to be mediated, at least partially, by the inducible nitric oxide synthase (iNOS) or neuronal nitric oxide synthase (nNOS) induction [16].

Also of interest is the nootropic potential of agomelatine. Preclinical studies investigating the efficacy of agomelatine on learning and memory have shown that it increases hippocampal neurogenesis [17–19]

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and improves cognitive processes [20,21]. This nootropic effect has been suggested to be associated with an increase in the levels of neuroplasticity-related molecules, such as brain derived neurotrophic factor (BDNF), neural cell adhesion molecule (NCAM), activity-regulated cytoskeletal-associated protein (Arc), and fibroblast growth factor-2 (FGF-2), which play important roles in cognitive processes in the CNS [17,19,20,22,23].

Taken together, all these studies show that agomelatine has widespread pharmacological activity potential in the CNS. However, very little is identified about the effect of this molecule on neuronal pathways carrying painful impulses. This, as well as the anti-nociceptive effect potential of various other antidepressant medications [24–26], prompted us to examine the antinociceptive activity of agomelatine in nociceptive pathways by using acute nociceptive experimental models. Furthermore, we examined the probable involvement of opioidergic system in the pharmacological effect.

2. Materials and methods

2.1. Experimental animals

Tests were performed using Swiss male mice (30–35 g). The animals were housed in an air-conditioned room (24 ± 1 °C) with a 12-h light and 12-h dark cycle. The experimental protocol of this study was approved by the Local Ethical Committee on Animal Experimentation of Anadolu University, Eskişehir, Turkey.

2.2. Drugs and treatments

Agomelatine (Valdoxan®, Servier, Wicklow, Ireland) is commercially available. Acetic acid was obtained from Merck (Darmstadt, Germany) and naltrindole hydrochloride from Tocris Cookson (Ballwin, MO, USA). Morphine sulfate, naloxonazine dihydrochloride, nor-binaltorphimine dihydrochloride, and naloxone hydrochloride dehydrate were acquired from Sigma-Aldrich (St Louis, MO, USA).

Animals were randomly distributed into four groups: control group, reference group (morphine sulfate, 10 mg/kg) [27], agomelatine-treated test groups (20, 40, and 60 mg/kg) and antagonist pretreated (naloxonazine, 7 mg/kg; nor-binaltorphimine, 1.03 mg/kg; naltrindole, 0.99 mg/kg; naloxone, 5.48 mg/kg) test groups. Agomelatine and naloxonazine were administrated to mice via intragastric and subcutaneous routes, respectively. On the other hand, morphine sulfate, nor-binaltorphimine, naltrindole, and naloxone were administered by intraperitoneal injections. The doses were chosen according to the previous experiences of our laboratory and to data previously reported for mice [27–29].

2.3. Assessment of anti-nociceptive activity

2.3.1. Tail-clip test

The tail-clip test is a frequently preferred experimental method for assessing the antinociceptive activity of drugs against mechanical nociceptive stimuli [30,31]. In this test, a mechanical noxious stimulus was applied by using a metal artery clamp. After applying the clamp to the tail of mouse, the "response latency" (the time until the mouse bites the clamp) of the mouse was recorded using a stopwatch. Elongation of response latency is considered a correlate of antinociception.

To avoid false positive measurements, before the experimental sessions, a "sensitivity test" was performed and only mice responding within 10 s were selected for the tests. The noxious stimulus was not applied for longer than 10 s (cut-off time) to prevent probable damage to the mouse's tail [32,33].

2.3.2. Hot-plate test

The hot-plate test is a frequently performed experimental method for assessing the antinociceptive activity of drugs against thermal nociceptive stimuli [34]. In this test, a thermal noxious stimulus was applied by using a hot-plate analgesiometer (Ugo Basile, No. 7280, Italy), which was fixed at 55 ± 1.0 °C. The "response latency" was recorded as the time until the mouse licked its forepaws or jumped. Elongation of "response latency" is considered a correlate of antinociception.

To avoid false positive measurements, before the experimental sessions, a "sensitivity test" was performed and only animals responding within 15 s were selected for the tests. The noxious stimulus was not applied for longer than 30 s (cut-off time) to prevent probable damage to the paw of the mouse [32,33].

The tail-clip and hot-plate latencies were converted to percentage of the maximum possible effect (MPE%) by using the following equation [32,33]:

$$\text{MPE}\% = [(\text{postdrug latency} - \text{predrug latency})/(\text{cut off time} - \text{predrug latency})] \times 100$$

2.3.3. Acetic acid-induced writhing test

The acetic acid-induced writhing test is a frequently preferred experimental method for evaluating the antinociceptive effects of drugs against noxious chemical stimuli [35]. In this test, a noxious chemical stimulus was applied by injecting 0.6% acetic acid solution (10 ml/kg body weight). I.p. administration of acetic acid, a chemical nociceptive stimulus, triggers writhing behavior (contraction of the abdominal muscles accompanied by an extension of the hind limbs and elongation of the body) [36]. Five minutes later the acetic acid administration, the quantity of writhing behaviors throughout the following 10 min was counted. Reduction in the number of writhing behaviors is considered a correlate of antinociception [32,33]. The antinociceptive effect was quantified by the following formula:

$$\text{Protection}\% = [(\text{control mean} - \text{treated mean})/\text{control mean}] \times 100$$

Tests were started 30 min after the i.p. injection of morphine and 60 min after the p.o. administration of vehicle or agomelatine.

2.4. Role of opioid mechanisms in the anti-nociceptive effect of agomelatine

The potential participation of the opioid system in the acute antinociceptive activity of agomelatine was investigated by utilizing an antagonism paradigm. The mechanistic study was conducted with the 40 mg/kg dose of agomelatine, since this dose appeared to induce maximum antinociceptive effect.

To elucidate the participation of opioid system to the observed antinociceptive activity, naloxone (non-selective opioid receptor antagonist, 5.48 mg/kg) was used. Mice were pretreated with naloxone 15 min before the vehicle or agomelatine administrations. 60 min later, the nociceptive tests were conducted [27].

As a next step, the potential contribution of opioid receptor subtypes was examined using specific opioid receptor subtype antagonist compounds. Naloxonazine (μ-opioid receptor selective antagonist, 7 mg/kg), naltrindole (δ-opioid receptor selective antagonist, 0.99 mg/kg) and nor-binaltorphimine (κ-opioid receptor selective antagonist, 1.03 mg/kg) were used to explore the probable role of μ, δ, and κ receptors in antinociceptive effect of agomelatine. Similar to the naloxone protocol, mice were pretreated with these antagonist 15 min before the vehicle or agomelatine administrations [27].

2.5. Assessment of motor coordination

Rota-rod apparatus (Ugo Basile, No. 47600, Italy) was used to estimate the probable influence of agomelatine on motor coordination of animals [37]. In the training period, 3 trials were conducted for 3 consecutive days. Animals that were able to stay on the rotating (16 revolutions/min) bar more than 180 s were chosen for the tests.