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The analgesic effects of peripheral and central administration of melatonin in rats

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Abstract

To explore the site and mechanism of the analgesic action of melatonin, the present study was designed to evaluate the analgesic effects of intraperitoneal (i.p.) and intracerebroventricular (i.c.v.) administration of melatonin, and to investigate the effect of i.c.v. naloxone on the analgesic effect induced by i.p. melatonin in rats. Antinociception was determined by tail-flick latency to hot water at 50°C. On i.p. administration, melatonin (30, 60 and 120 mg/kg) produced the antinociceptive effect in a dose-dependent manner, with an A_{50} of 72.8 mg/kg. Administered i.c.v., melatonin (0.25, 0.5 and 1 mg/kg) also resulted in dose-dependent antinociception, with an A_{50} of only 0.693 mg/kg. Injected i.c.v. to rats, 10 μg of naloxone antagonized significantly the antinociceptive effect induced by i.p. melatonin. It is concluded that melatonin has an analgesic effect in rats and the central nervous system (CNS) may be the primary site for melatonin to elicit the response, and the effect of melatonin is related to the central opioid system.

Keywords: Melatonin; Central nervous system; Analgesia; Naloxone

1. Introduction

Melatonin is the main hormone secreted by the pineal gland. It was isolated in 1958 (Lerner et al., 1958). This indole compound (*N*-acetyl-5-methoxytryptamine) is derived from serotonin after two biochemical steps. It has been implicated in some pharmacological effects including sedative/hypnotic, anticonvulsant activity, etc. (Sugden, 1983; Geoffriau et al., 1998). In particular, melatonin administered intraperitoneally (i.p.) has been shown to possess a potent and long-lasting antinociceptive effect in mice and rats, suggesting that it produces analgesia (Lakin et al., 1981; Golombek et al., 1991; Yu et al., 1999b).

However, the site and mechanism of action of melatonin to induce analgesia remain to be clarified. Melatonin receptors are found in both the central nervous system (CNS) and peripheral tissues (Vanecek, 1998). Melatonin is known to exert its effects through melatonin receptors. Relatively abundant melatonin receptors are found in several brain regions, particularly the hypothalamus (Stankov et al., 1991a,b; Morgan et al., 1994). Moreover, Pang and Brown (1983) have reported that melatonin in the brain is unevenly distributed, with a high level in the hypothalamus, and that the ratio of its concentration in whole brain to that in serum is about 9:1 during the dark period and 3:1 during the light period in rats. Melatonin can penetrate the blood–brain barrier. Therefore, it is logical to assume that the brain may be one of the most important sites for melatonin to exert an analgesic effect. To clarify this matter, we evaluated the analgesic effects of i.p. and intracerebroventricular (i.c.v.) administration of melatonin in rats. Furthermore, others and we have previously shown that peripheral administration of naloxone may blunt the analgesic effect induced by melatonin (Lakin et al., 1981; Golombek et al., 1991; Yu et al., 1999b). Several observations point to a significant interaction between melatonin and opioid peptides in the brain (Kumar et al., 1982; Xu et al., 1995). It was of interest to determine whether melatonin-induced analgesia was related to the central opioid system. The present study investigated the effect of i.c.v. naloxone on melatonin-induced analgesia.

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2. Materials and methods

2.1. Animals

Experiments were performed on male Sprague–Dawley rats weighing 180–230 g which were supplied by the Experimental Animal Center, Shanghai Medical University, China. The animals were housed in groups of four, with free access to standard rat diet and tap water in a room with 12:12 h light/dark cycle (lights on from 08:00 to 20:00 h). Each rat was used in one experiment only. All the protocols in the present study were approved by the local ethics committee in Shanghai, China.

2.2. Drugs

Melatonin was purchased from Sigma, USA. It was dissolved in 8% ethanol saline (v/v) immediately before use. Naloxone hydrochloride was obtained from Sigma, and was dissolved in 0.9% NaCl solution (normal saline).

2.3. Intracerebroventricular injection

Under pentobarbital anesthesia (35 mg/kg, i.p.), the rat was immobilized on a stereotaxic apparatus. The skin was cut along the midline of the skull. A cotton swab soaked with 3% H_2O_2 was used to abrade the tissue until the skull was exposed. A stainless steel cannula of 0.8 mm outer diameter was fixed on the skull stereotaxically (1 mm posterior to the bregma, 2.0 mm lateral to the midline and 4.0 mm from the surface of the brain) with dental resin, directed to one side of the lateral ventricle (Wang et al., 1999). Experiments with i.c.v. injection were performed 72–96 h after surgery. A stainless steel injection tube was inserted into the cannula with the lower end protruding 0.5 mm beyond the lower end of the cannula to reach the lateral ventricle. Then 25 μl melatonin solution or 10 μl naloxone hydrochloride solution was injected into the lateral ventricle within 1 min. Control groups were injected with the respective vehicle. At the end of each experiment, stainless steel tubing of the same size as the injection tube was inserted into the guide cannula, 10 μl of a solution of pontamine sky blue was injected. Ten minutes later, the rat was decapitated and its brain was removed to be cut for checking the validity of the i.c.v. injection. Data from animals in which the cannula had been incorrectly placed were discarded.

2.4. Measurement of antinociception

Treatment of the rats conformed to the guidelines of the International Association for the Study of Pain (Zimmermann, 1983). The pain sensitivity of rats was measured with the hot water tail-flick test (Wang et al., 1999). The room temperature was kept at 22 ± 1°C. The pain threshold was measured from 13:00 to 16:00 h (during the mid-light period). The rats were lightly restrained in a wooden holder and allowed to adapt to the holder. The tail flick latency was determined by placing the distal part of the tail (5.5 cm) in a beaker containing water maintained at 50°C. Baseline tail flick latency was defined as the mean of three determinations at 10 min intervals before drug injection. Only those rats with the baseline latency within the range of 4–7 s were used for further studies. Following drug administration, tail flick latency was measured at selected time intervals. The antinociceptive effect in the above test is presented either as latency or calculated as percentage change of tail flick latency from the baseline level according to the formula: percentage change = [(postdrug latency − predrug latency)/predrug latency] × 100. The data were expressed as the means ± S.E.M. and analyzed statistically with a one-way ANOVA (analysis of variance) followed by the Student–Newman–Keuls test. A level of $P < 0.05$ was accepted as an indication of significance.

2.5. Treatment schedules

In order to evaluate the analgesic effect of i.p. administration of melatonin, the rats were divided into four groups of eight each, and given an i.p. injection of vehicle, melatonin 30, 60 and 120 mg/kg, respectively. To assess the analgesic effect of i.c.v. administration of melatonin, 32 rats were divided into four equal groups, and given i.c.v. vehicle 25 μl, melatonin 0.25, 0.5 and 1 mg/kg, respectively. In an attempt to determine whether naloxone antagonized the analgesia induced by i.p. melatonin, 16 rats were divided into two equal groups, and given an i.p. injection of 120 mg/kg of melatonin. Five minutes later, one group was given an i.c.v. injection of 10 μg of naloxone, and the other group one of normal saline, 10 μl, as control. Moreover, to assess whether naloxone itself affects the tail flick latency, five rats were given an i.c.v. injection of 10 μg of naloxone.

3. Results

3.1. Antinociceptive effects of i.p. melatonin

The mean baselines of tail flick latency in the four groups of rats ($n = 8$, each) were 6.12 ± 0.38, 5.91 ± 0.52, 5.88 ± 0.57 and 5.72 ± 0.47 s, respectively. There were no significant differences ($P > 0.05$) between the mean baselines of tail flick latency in the vehicle-treated and melatonin-treated rats. As shown in Fig. 1, melatonin produced dose-dependent antinociception following its i.p. administration. In the groups with larger doses (60 and 120 mg/kg), the effective antinociception started 15 min after melatonin administration, reached a peak after 30 min and lasted more than 100 min. The dose–response line for the i.p. administration of melatonin at the time of peak effects,

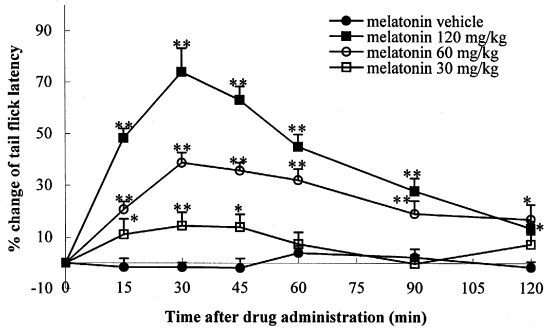

Fig. 1. Antinociceptive effect of i.p. melatonin in rats. Antinociception was determined by tail flick latency to hot water at 50°C. Ordinate shows percentage changes of tail flick latency from baseline level. All points represent the mean and S.E.M. from eight rats. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. vehicle-treated group.

30 min, is presented in Fig. 3A, with A_{50} (the dose of 50% increase of tail flick latency) calculated to be 72.8 mg/kg (95% C.L. 40.7–234.2 mg/kg).

3.2. Antinociceptive effects of i.c.v. melatonin

The mean baselines of tail flick latency in the four groups of rat ($n = 8$, each) were 6.16 ± 0.25, 6.05 ± 0.48, 6.03 ± 0.38 and 6.07 ± 0.42 s, respectively. There were no significant differences ($P > 0.05$) between these data. The i.c.v. administration of melatonin resulted in dose-dependent antinociceptive effects (Fig. 2). In the group with the high dose (1 mg/kg), the antinociception started 10 min after melatonin administration, reached a peak after 30 min and lasted more than 80 min. The dose–response line for the i.c.v. administration of melatonin was plotted at the time of peak effects, 30 min, and is shown in Fig. 3B, with A_{50} calculated to be 0.693 mg/kg (95% C.L. 0.406–4.393 mg/kg), which is about 1/105 of the A_{50} for melatonin administered i.p.

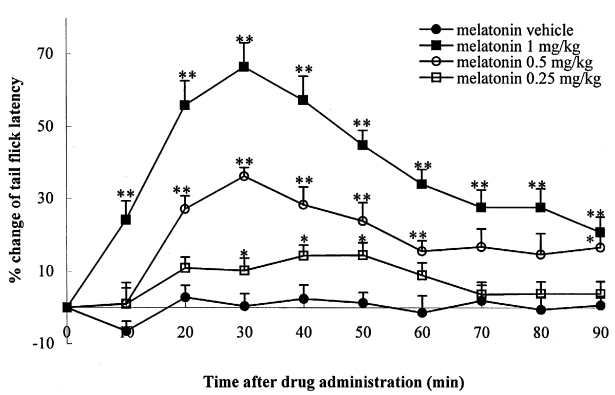

Fig. 2. Antinociceptive effect of i.c.v. melatonin in rats. Antinociception was determined by tail flick latency to hot water at 50°C. Ordinate shows percentage changes of tail flick latency from baseline level. All points represent the mean and S.E.M. from eight rats. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. vehicle-treated group.

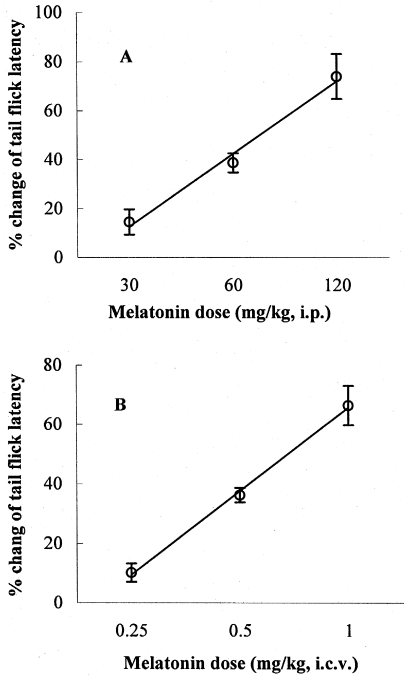

Fig. 3. Dose–response lines for melatonin given i.p. (A) or i.c.v. (B) to rats. Antinociception was determined by tail flick latency to hot water at 50°C. Ordinate shows percentage changes of tail flick latency 30 min after melatonin administration. All points represent the mean and S.E.M. from eight rats. The A_{50} of i.p. administration of melatonin was 72.8 mg/kg; the A_{50} of i.c.v. administration of melatonin was 0.693 mg/kg.

3.3. Effect of i.c.v. naloxone on the antinociceptive effect induced by i.p. melatonin

The mean baselines for tail flick latency in the two groups ($n = 8$ each) were 6.14 ± 0.41 and 5.99 ± 0.49 s, without a significant difference ($P > 0.05$). As shown in Fig. 4, the antinociceptive effect of melatonin was antagonized significantly by naloxone, starting 10 min after naloxone administration and lasting more than 45 min. The

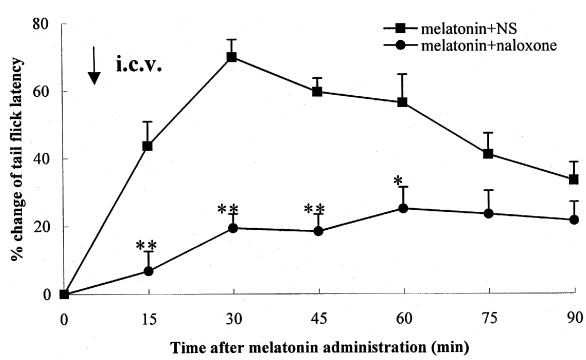

Fig. 4. Effect of i.c.v. naloxone (10 μg) on the antinociceptive effect induced by i.p. melatonin (120 mg/kg). Naloxone or NS (normal saline) was given i.c.v. 5 min after melatonin administration, as indicated by the arrow directed downward. Antinociception was determined by tail flick latency to hot water at 50°C. Ordinate shows percentage changes of tail flick latency from baseline level. All points represent the mean and S.E.M. from eight rats. $^{*}P < 0.05$; $^{**}P < 0.01$ vs. melatonin + NS group.

increases in tail flick latency 30 min after the i.p. administration of melatonin were 70.1 ± 5.1% and 19.4 ± 4.2% in the saline-treated and the naloxone-treated groups, respectively. Moreover, it was found that naloxone (i.c.v. 10 μg) itself did not alter tail flick latency significantly over the 90 min observation period (data not shown).

4. Discussion

A number of behavioral studies have shown that melatonin administered peripherally exerts an antinociceptive action against thermal and chemical stimuli in mice (Lakin et al., 1981; Sugden, 1983; Golombek et al., 1991). We have also observed (Yu et al., 1999b) that the i.p. injection of melatonin resulted in dose-dependent antinociception in rats and mice in a nociceptive test, electrical stimulation vocalization. In the present study, a similar antinociceptive effect induced by i.p. administration of melatonin was obtained in rats. Taken together, these results indicate that melatonin produce potent and long-lasting analgesia. We have shown recently (Yu et al., 1999a) that melatonin possessed an analgesic effect without producing physical dependence in mice. Anecdotal evidence suggests that very large doses (up to 6 g) of melatonin can be safely given to human subjects without any apparent side-effects ((Lerner and Nordlund, 1975). Sugden (1983) has reported that melatonin had very low acute toxicity when administered to mice and rats by various routes (i.p., p.o., s.c., i.v.). It thus seems that melatonin may have potential clinical value as a new and effective analgesic. In fact, it was recently reported (Maestroni, 1993; Leone et al., 1996) that using melatonin to treat patients with cluster headache might lower their pain indexes, without obvious toxic reaction or side effect.

Several studies demonstrated the existence of a diurnal rhythm in the latency of mice to respond to a pain stimulus, with maximal latencies being found at night (Lakin et al., 1981; Ying and Huang, 1990). Particularly, it was reported (Golombek et al., 1991) that melatonin exhibited a time-dependent analgesic effect, this effect being maximal at the light–dark transition and minimal approximately at the mid-light period. In view of the above observations, we performed all the measurements of pain threshold at the same time, from 13:00 to 16:00 h (approximately during the mid-light period). At the experimental time, we observed that melatonin produced a dose-dependent analgesic effect, with an A_{50} of 72.8 mg/kg upon i.p. administration. This effective dose was similar to that assessed at the mid-light period (Lakin et al., 1981), but much higher than the effective dose evaluated during the light–dark transition (Golombek et al., 1991). There are significant differences in the minimal effective doses of melatonin for producing analgesia, with a range of 20–200 mg/kg (Lakin et al., 1981; Sugden, 1983; Golombek et al., 1991; Yu et al., 1999b). Apart from differences in experimental setup such as animal species and nociceptive tests, a major factor in these differences between results from different laboratories is the time when melatonin was administered. In view of the chronobiological characteristics of melatonin-induced analgesia, it seems to suggest that melatonin administered in the late evening may be more efficacious in the treatment of pain.

To assess the effects of central and peripheral administration of a drug and compare its potency may be of some general use for studying the central site of drug action. In the present study, we observed that i.c.v. melatonin resulted in dose-dependent antinociception, with an A_{50} of only 0.693 mg/kg that is about 1/105 of the A_{50} for i.p. melatonin. Moreover, we found that the i.c.v. administration of naloxone counteracted significantly the antinociceptive effect induced by i.p. melatonin. These results suggest that the CNS may be the primary site for melatonin to exert its analgesic effect. Several facts support this suggestion: (a) the i.c.v. administration of luzindole, a selective MT2 melatonin receptor antagonist, completely antagonizes the antinociceptive effect induced by i.p. melatonin (Yu et al., 2000b), (b) relatively abundant melatonin receptors have been found in several brain regions (Stankov et al., 1991a,b; Morgan et al., 1994), (c) melatonin in the brain is unevenly distributed and the ratio of its concentration in the whole brain to that in the serum is about 9:1 in the dark period and 3:1 in the light period in rats (Pang and Brown, 1983). In addition, it was reported Lakin et al., 1981; Ying and Huang, 1990) that both basal nociceptive threshold and meperidine or morphine analgesia in mice followed a day/night rhythm, and that this rhythm was not evident in pinealectomized mice. The above findings suggest that the endogenous melatonin in the CNS may have a functional role in pain regulation.

The mechanism underlying the melatonin-induced analgesia is obscure. Some reports Lakin et al., 1981; Golombek et al., 1991; Yu et al., 1999b) claimed that the peripheral administration of naloxone blunted the analgesic response to melatonin. However, another study (Sugden, 1983) could not confirm this effect. In the present study, while the i.c.v. injection of 10 μg of naloxone to rats itself had no significant influence on the pain threshold, which is consistent with the data reported by Woolf (1980), it did antagonize the melatonin-induced analgesic effect. This result supports the existence of a naloxone-reversible effect of melatonin-induced analgesia, and can be regarded as strong evidence suggesting that the analgesic effect of melatonin be related to the central opioid system. In this case, how does the relationship between melatonin and the central opioid system affect analgesia? It is well known that β-endorphin in the brain, one of the endogenous opioid peptides, plays an important role in the endogenous antinociceptive system (Akil et al., 1984). The studies about melatonin and the release of β-endorphin in the brain should be taken into account. It was reported (Xu et al., 1995) that the β-endorphin concentration in the mouse

hypothalamus was significantly decreased 30 min after melatonin administered i.p. In our recent study (Yu et al., 2000a), it was found that melatonin may promote the release of β-endorphin from the periaqueductal gray in rats. Taken together, results of these studies indicate that melatonin promotes the release of β-endorphin in the brain, which may be one of the mechanisms of action of melatonin to induce analgesia.

In summary and conclusion, our findings show that melatonin has an analgesic effect in rats and the CNS may be the primary site for melatonin to exert it. Furthermore, this work provides evidence that the analgesic effect of melatonin is related to the central opioid system.

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