Analgesic Effect of Melatonin in Mice

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Abstract

Background: Melatonin is the main hormone secreted by the pineal gland. This indole compound (N-acetyl-5-methoxytryptamine) is derived from serotonin after two biochemical steps. Melatonin has been implicated in some pharmacological effects including sedative/hypnotic, anticonvulsant activity and others. The aim of this study was to investigate the antinociceptive effect of different doses of melatonin administered i.p. to mice, and then, to find the dose-response line of melatonin in mice as analgesic agent.

Methods: The dose response effect of melatonin (10, 50, and 100mg/kg) were assessed against control using tail flick test in mice as a model of nociceptive pain. In this model, all doses of melatonin were given intraperitoneally 15 min before immersion of tail in hot water 50°C, and Tail Flick Latency was measured before, and after (15, 30, 60 and 120 min) administration of melatonin.

Results: Administration of melatonin i.p. to mice significantly $P \leq 0.05$ increase tail flick latency in melatonin treated groups after 30 min and 60 min compared to baseline values; while after 120 min, administration of melatonin produce significant and dose dependent antinociceptive effect following its i.p. administration. The percentage increase in tail flick latency produced by i.p. administration of melatonin doses of 10, 50, and 100 mg/kg were 86.59%, 156.05% and 169.19% respectively when compared to baseline values.

Conclusions: The present study showed that melatonin produces analgesic effect in a dose dependent manner in mice, further studies are required to know the exact mechanism by which melatonin exerts this analgesic effect.

Keywords: melatonin, analgesia, pain

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Introduction

Melatonin is the main hormone secreted by the pineal gland. This indole compound (N-acetyl-5-methoxytryptamine) is derived from serotonin after two biochemical steps [(1).] Melatonin has been implicated in some pharmacological effects including sedative/hypnotic, anticonvulsant activity and others [(2).] In particular, melatonin has been shown to possess a potent and long lasting antinociceptive effect in rodents, suggesting that it produce analgesia [(3).] The site and mechanism of action of melatonin to produce analgesia remain to be clarified. Melatonin receptors are found in both the central nervous system and peripheral tissues [(4).] Melatonin is known to exert its effects through melatonin receptors; relatively abundant melatonin receptors are found in several brain regions, particularly the hypothalamus; moreover 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 dark period and 3:1 during the light period [(5).]

Melatonin can penetrate the blood-brain barrier; therefore, brain may be one of the most important sites for melatonin actions. It has been suggested that melatonin is a strong synchronizer of a broad spectrum of activities ranging from alteration of skin color to resetting the circadian clock in mammals, thermoregulation, communication of time of day and season between fetus and mother, regulation of appetite and regulation of altered environmental conditions [(6).]

Furthermore, it has been repeatedly reported that melatonin act as antioxidant [(7);] it functions as a scavenger of hydroxyl, peroxyl, and superoxide radicals. In the brain tissue, melatonin effectively protects against lipid peroxidation; it may act also by stimulating enzymes involved in the antioxidative defense cascade like glutathione peroxidase [(8).]

A possible analgesic effect of melatonin has been suggested in patients suffering less from pain during the night when melatonin levels are high, than during the day. Prolong latencies of pain threshold have been measured in healthy subjects during the dark phase of the photoperiod; while pinealectomy abolishes this dark phase analgesia [(9)] and application of melatonin is able to restore it [(10).]

The aim of this study was to investigate the antinociceptive effect of different doses of melatonin administered i.p. to mice, and then, to find the dose- response line of melatonin in mice as analgesic agent.

Methods

Animals: 28 albino mice obtained from animal house in college of veterinary medicine – Baghdad University, weighing 20-25gm were

used in the experiments. The animals were housed in standard stainless steel cages at room temperature with 12-12 hour light dark cycle. The mice were randomly distributed into 4 groups of seven as control and test subjects. All animals had access to food and water freely through out the experiments. All experiments were performed considering all ethical circumstances. For antinociception recording, mice were allowed to acclimatize for 30 minutes before intraperitoneal injection.

Preparation of doses: the doses of 10, 50, and 100mg/kg of melatonin as crude powder (Amon Co, Egypt) was dissolved in 8% ethanol saline (v/v) immediately before use to produce the required strength.

In the present study, animals were allocated into 4 groups, seven of each, the first group treated with vehicle only and served as control; second, third and fourth groups treated with melatonin 125,250, and 500mg/kg respectively.

Measurement of antinociception: in this study, the pain sensitivity of mice was measured with hot water tail flick test (11), the pain threshold was measured during the mid –light period. The tail flick latency was determined by placing the distal part of the tail in a beaker containing water maintained at 50°C. Baseline tail flick latency was the value before administration of any drug. Following drug administration tail flick latency was measured at selected time intervals of 15 min, 30 min, 60min and 120 min respectively.

Statistical Analysis: data were expressed as the mean ± SE; and analyzed statistically with student t-test, P-value ≤ 0.05 were considered significant.

Results

The data obtained in this study showed that there were no significant differences between the baseline values of the four studied groups of mice (n=7,each) concerning the tail flick latency, which were 5.22±1.2, 4.92±0.95, 4.21±1.7 and 5.42±1.5 respectively.

Administration of melatonin doses of 10 and 50 mg/kg produce non-significant change in tail flick latency in mice compared to vehicle treated group after 15 min; on the other hand, administration of melatonin in a dose of 100mg/kg i.p. to mice significantly increase tail flick latency after 15 min by 66.79% compared to base line value.

Results in table (1) indicate that administration of melatonin i.p. to mice significantly P ≤ 0.05 increase tail flick latency in melatonin treated groups after 30 min and 60 min compared to baseline values; while after 120 min as shown in table (1) and figure (1) , administration of melatonin produce significant and dose dependent antinociceptive effect following its i.p. administration. The percentage increase in tail flick latency produced by i.p. administration of melatonin doses of 10,50, and 100 mg/kg were 86.59%, 156.05% and 169.19% respectively when compared to baseline values.

Table 1: Effect of melatonin (10, 50 and 100mg/kg) on latency to withdrawal tail in tail flick test in mice.

Treatment	Latency to withdrawal tail (sec)				
	0 min	**15 min**	**30 min**	**60 min**	**120 min**
Control	5.22±1.2	5.3±1.3	5.1±2.1	5.5±1.82	5.08±1.92
Melatonin 10mg/kg	4.92±0.95	6.74±1.42	9.13±2.75*	8.19±1.4*	9.18±2.3*
Melatonin 50mg/kg	4.21±1.7	5.34±1.42	7.83±1.73*	8.57±2.33*	10.78±3.6*
Melatonin 100mg/kg	5.42±1.5	9.04±2.21*	12.06±4.4*	14.98±1.8*	14.54±5.56*

Results represent mean ± SE; * P ≤ 0.05 considered significant change as compared to control.

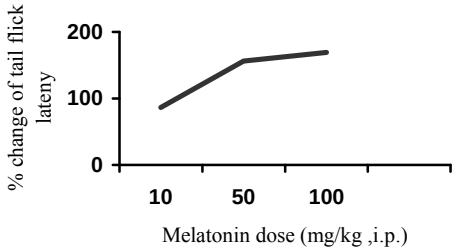

Figure (1): Dose –response line for melatonin given i.p. to mice.

Antinociception was determined by tail flick latency to hot water 50°C. Ordinates shows percentage changes to tail flick latency 120 min after melatonin administration. All points represent mean ± SE from seven mice.

Discussion:

Animal studies have suggested a correlation between melatonin levels and the intensities of responses to painful stimuli; in addition, nociceptive thresholds and latencies to painful stimuli reflect a diurnal fluctuation that is the inverse of circadian rhythm of melatonin (12). It has been found that nociceptive threshold increased during the dark , and decreased during the light (13). Darkness is known to be the time of peak synthesis and secretion of melatonin (14). Rodents in continuous dark had enhanced nociceptive threshold similar to dark – time threshold of control rodents under 12-hr light/dark periods (15). Pinealectomy eliminates the differences between light-time and dark-time latencies of responses to painful stimuli in rodents (16).

A number of behavioral studies have shown that melatonin administration exerts an antinociceptive action against thermal and chemical stimuli in mice (17). It has been also observed that the i.p. injection of melatonin resulted in antinociception in rats and mice in nociceptive test; in the present study, similar antinociceptive effect induced by i.p. administration of melatonin was obtained in mice, the increase in tail flick latency was significant and dose dependent. These results indicate that melatonin produce potent and long lasting analgesia. It has been reported that melatonin possessed analgesic effect without producing physical dependence in mice; and that very large doses (up to 6 g) of melatonin can be safely given to human subjects without any apparent side effects (18).

Thus, it seems that melatonin may have potential clinical value as new and effective analgesic agent. Recently, it was reported that melatonin is used to treat patients with cluster headache and migraine (19), also melatonin used to treat the painful condition that associate with irritable bowel syndrome (20).

The mechanism of melatonin-induced antinociception however is largely unknown. It is generally assumed that melatonin exert responses through activation of melatonin receptor. Others reported that melatonin reduced the level of oxidative stress end product that is previously increased by painful stimuli, i.e. melatonin exert its antinociceptive effect through its antioxidant activity (21). The other potential mechanism by which melatonin exerts its antinociceptive effect was potentiation of $GABA_A$ receptor mediated current (22). The exact mechanism of action by which melatonin produce analgesic effect need further deep investigated studies.

In conclusion, the present study showed that melatonin produces analgesic effect in a dose dependent manner in mice, further studies are required to know the exact mechanism by which melatonin exerts this analgesic effect.

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