Melatonin replacement nullifies the effect of light-induced functional pinealectomy on nociceptive rhythm in the rat

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Abstract:

Rats maintained on a 12 h daily photoperiod (12:12 LD cycle), exhibited a diunal variation in sensitivity to both heat-elicited and pressure-elicited pain, with low sensitivity at 2 h before the end of the scotophase and higher at 4 h after the onset of photophase. Functional pinealectomy induced by a single LL day effaced the baseline diurnal rhythm of sensitivity to pressure-elicited pain, and reversed that to heat-elicited pain. Oral administration of physiological doses of melatonin into functionally pinealectomized rats, nullified the effect of functional pinealectomy, restoring the normal baseline rhythms of both pressure-elicited and heat-elicited nociceptive responses. The role of melatonin in modulating nociception is discussed in light of an indoleaminergic-opioid system.

Keywords: Melatonin | Nociception | Diurnal rhythm | Functional pinealectomy

Article:

Following the discovery that light and darkness regulate both the biochemical activity (42,56) and endocrine capability (20) of the pineal gland, there has been considerable interest in the physiological and behavioral functions of this gland. Although once presumed to be a vestigial organ having no functional significance, the pineal gland is now recognized as an integral part of the vertebrate neuroendocrine system that modulates biorhythms (47). Melatonin (N-acetyl-5-methoxytryptamine), an indoleamine mainly synthesized in the pineal gland, has generally been considered as the principal pineal hormone (43,46,57). In most vertebrate species, the circulating as well as the pineal levels of melatonin exhibit a circadian pattern, with peak levels in the scotophase. Diurnal variations in pain sensitivity have also been reported in several species including mice (9,12,15,24,25,30), rats (5,36,41,48), hamsters (40), and humans (10). While such day–night rhythm of nociceptive sensitivity is suspected to be related to changes in opioid activity [see review: Henry (16)], the pineal gland has also been implicated in the modulation of the diurnal rhythm of nociception, at least in mouse (24,30) and rat (5,36,41,48). In the mouse,

melatonin has been demonstrated to have an analgesic property (15,24,30). Using two different methods of nociceptive sensitivity tests (tail-flick test and hot-plate test), Bar-Or and Brown (5) have demonstrated that in rats maintained on a 12 h daily photoperiod, pain sensitivity was the lowest when tested 2 h before lights were on (-2 h) and highest 4 h into the photophase (+4 h). Following surgical pinealectomy or light-induced functional pinealectomy, the magnitude of difference in hot-plate response latencies between the two time points (i.e. -2 h vs. +4 h) was found to be reduced (5), implying that melatonin could be involved in modulating the diurnal rhythm of nociception in rats. Nevertheless, the effect of melatonin on nociception in rats has not yet been directly demonstrated. The present study was carried out to determine whether or not melatonin replacement at physiological levels in functionally pinealectomized rats can restore the normal diurnal rhythm of nociception.

METHOD

Male Wistar rats, each weighing between 100 and 120 g, were obtained from a local supplier and were individually housed in cages located in a controlled environmental chamber. The rats were given water and commercial rat feed ad lib, and were acclimated to a 12 h daily photoperiod (lights on at 0930 h and off at 2130 h) and an ambient temperature of 22 ± 0.5°C for 4 weeks prior to the commencement of the experiment. Lighting was provided by cool white fluorescent bulbs that supplied an illumination of approximately 110μW/cm^2 (according to the reading taken on an Opikon radiometer with broad spectrum head and infrared filter). Red photosafe bulbs were used to allow visualization while carrying out testing procedures in the dark. In rats maintained under similar conditions, a distinct circadian rhythm of melatonin concentration in both pineal and serum (18, 19) and of the melatonin metabolite 6-sulphatoxymelatonin in urine (6) has been observed previously in this laboratory.

Tests of Nociception

The following two methods were employed to evaluate nociception.

Tail-flick test. A custom-built (by Colin Ikeson, McMaster University) tail-flick apparatus was used to measure the sensitivity to noxious stimulus. Radiant heat provided by a projection bulb (ELH, 300 W, 120 V), located 8 cm above the dorsum of the rat tail, served as the nociceptive stimulus. The rat was held on a special platform beneath which a photoelectric cell was housed. The tail was lightly secured between two flat Plexiglas blocks so that the light fell about 7 cm rostral to the tip of the tail. The projection bulb was automatically turned on when the attached timer was activated, and both were simultaneously shut off when the rat's tail moved in response to the heat. The rat's reaction threshold to the noxious stimulus as measured by the time required (seconds elapsed) for the tail to flick was automatically displayed on the LCD display screen of the timer.

Paw pressure technique. The technique involved the use of a mechanical device to apply a gradually increasing pressure on the rat paw. The pressure was increased until the rat attempted to pull the paw away or until vocalization occurred. The pressure reading was then recorded. The apparatus used was made in our laboratory. It consisted of a sphygmomanometer connected to a 20 ml syringe by an inelastic flexible tubing. The syringe was clamped vertically with the

plunger pointing downward. A polypropylene conical microcentrifuge tube was glued to the end of the plunger so that the blunt conical tip of the centrifuge tube pointed downward and formed the compression point.

Baseline diurnal rhythm of nociception: because Bar-Or and Brown (5) have earlier demonstrated that under 12:12 h light:dark (12:12 LD) cycle, the lowest and highest pain sensitivity in rats occur at -2 h and +4 h, respectively, only these two time points were examined in the present study. Both tail-flick and paw-pressure methods were employed. During weeks 5 and 6, the rats were subjected to the tests seven times, to accustom them to the testing procedures and to a possible confounding effect of habituation. Rats appeared to have become familiarized to handling by week 6. Experiments were commenced following 6 weeks of acclimation. Rats weighed approximately 225-250 g each, by this time. Measurements of tail-flick latency and paw pressure were recorded in 36 rats at both -2 h and +4 h.

Functional pinealectomy: in the context of the present experiment, functional pinealectomy refers to light-induced suppression of pineal activity for 1 day. This was achieved by exposing rats to light (as in photophase) during what would normally be the scotophase of their LD cycle. It introduced a single LL cycle in between the regular LD cycles. This exposure to light for 1 day has previously been shown to suppress pineal activity during that day (6). The same 36 rats tested to verify the baseline nociceptive rhythm were used for the functional pinealectomy study, 4 days later. All rats were subjected to functional pinealectomy. However, 18 of them were randomly selected and given melatonin with their drinking water so as to restore the circulating melatonin levels in the body. The remaining 18 rats were given vehicle solution for their drink, and were treated as controls. Neither tail flick response nor paw pressure response showed significant difference between the two groups prior to treatment.

Melatonin solution and dosage: the concentration of melatonin supplied in the drinking water was altered every 3 h during the 12 h functional pinealectomy period so a, to produce a circulating melatonin level that would mimic the natural endogenous melatonin level and rhythm observed under normal 12:12 LD cycle. Crystalline melatonin was initially dissolved in absolute ethyl alcohol and was further diluted with water to a final alcohol concentration of 0.1% (v/v). Melatonin drinks, with concentrations of 4 ng. 12 ng, 65 ng, and 4 ng per ml were given during the lst, 2nd, 3rd, and 4th 3 h periods of the 12 h functional pinealectomy phase, respectively. This regimen of oral dosage was considered adequate to generate a serum melatonin level and rhythm that would resemble the natural level and rhythm under a 12:12 LD cycle, because studies (22) in our laboratory have shown that the level and circadian rhythm of 6-sulphatoxymelatonin excreted under such oral melatonin regimen in functionally pinealectomized rats resembled that of rats maintained under a 12:12 LD cycle. Because this previous study (22) abo indicated that the control and melatonin groups did not differ significantly in the intake of drink, the drink consumption was not measured in the present study.

Statistical evaluation: data were analyzed by repeated measures analysis of variance. For multiple comparisons, Duncan's new multiple range test was employed. Results are expressed as mean ± SEM.

RESULTS

Rats acclimated for 6 weeks under 12:12 LD cycle and 22 ± 0.5°C ambient temperature exhibited a diurnal variation in the sensitivity to pain. With tail-flick test, the latencies were longer (i.e., lower pain sensitivity) at 2 h before the end of the scotophase (-2 h), and shorter (i.e., higher pain sensitivity) at 4 h after the onset of photophase (+4 h) (Fig. la). Results from paw-pressure tests were similar, showing lower pain sensitivity (i.e., greater compression required for response) at -2 h and higher sensitivity (i.e., lower compression required) at +4 h, in normal 12:12 LD cycle (Fig. 1b).

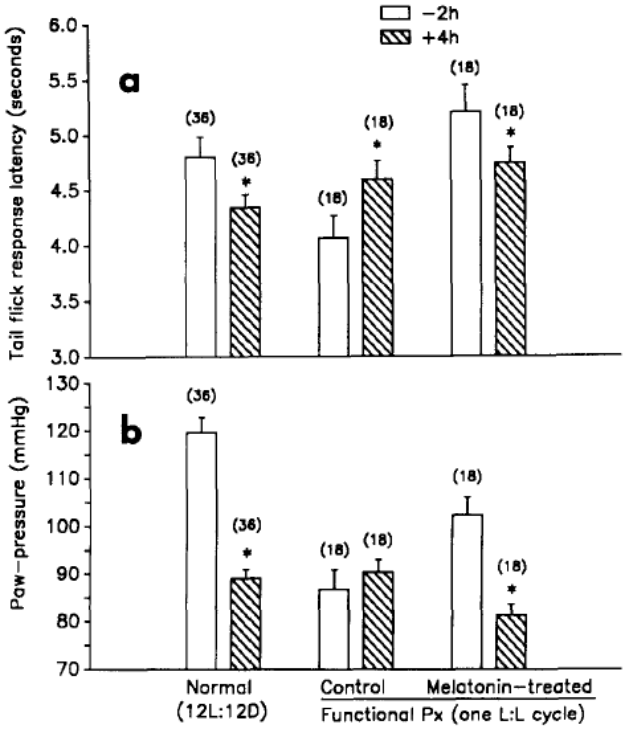

FIG. 1. (a) Radiant heat-elicited (tail flick) and (b) pressure-elicited (paw pressure) analgesic responses in rats. Means ± standard errors are shown. Figures in parentheses (above the bars) represent number of rats used. Normal refers to rats maintained on a 12:12 LD cycle. Functional Px refers to rats subjected to light-induced functional pinealectomy. -2 h and +4 h denote 2 h before and 4 h after the end of scotophase respectively (under functional Px, the scotophase refers to the one replaced by the photophase). ANOVA results for the tail flick response (functional Px): control vs. melatonin, $p = 0.008$; time, $p = 0.807$; interaction, $p = 0.002$. *$p <$ 0.05 when compared to -2 h group. ANOVA results for paw pressure response (functional Px): control vs. melatonin, $p = 0.408$; time, $p = 0.002$; interaction, $p < 0.0001$. *$p < 0.01$ when compared to respective -2 h group.

Under functional pinealectomy, there was no significant difference between the two time points (-2 h vs. +4 h) with respect to paw-pressure response (Fig. 1b), suggesting that the diurnal variation in pressure-elicited nociceptive response observed under normal 12:12 LD cycle is effaced under functional pinealectomy. Functional pinealectomy also disrupted the normal rhythm of the heat-elicited nociceptive response as indicated by tail-flick test. The rhythm showing longer tail-flick latency at -2 h and shorter latency at +4 h under the normal 12:12 LD

cycle was found reversed under functional pinealectomy, resulting in shorter latency at -2 h and longer at +4 h (Fig. 1a).

Oral administration of physiological doses of melatonin into functionally pinealectomized rats nullified the effect of functional pinealectomy, restoring the normal baseline rhythms of both heat-elicited (Fig. 1a) and pressure-elicited (Fig. 1b) nociceptive responses.

DISCUSSION

Using the tail-flick test, Rosenfeld and Rice (48) have demonstrated that in rats maintained under a 12 h daily photoperiod, the pain sensitivity to radiant heat was greater in the morning and afternoon, and lower later on. Pilcher et al. (41) subsequently carried out a 24 h mapping of the nociceptive sensitivities by testing different groups of animals at 2 h intervals over an entire 12:12 LD cycle. Their study revealed a bimodal rhythm of sensitivity to noxious heat (tail immersion method), while pressure-elicited nociceptive response exhibited only a simple diurnal rhythm, having the lowest sensitivity late in the scotophase. More recently, using tail-flick tests and hot-plate tests, Bar-Or and Brown (5) carried out a comprehensive study of pain sensitivity rhythms in rats subjected to various conditions, viz. 12:12 LD cycle, 1 day functional pinealectomy, and surgical pinealectomy. Their observation that under a 12:12 LD cycle the least sensitivity to pain was at -2 hand the greatest sensitivity was at +4 h, was consistent with the observation of Pilcher and co-workers (41) with regard to the pattern of sensitivity to pressure, but not tail immersion. Bar-Or and Brown (5) also demonstrated that the cues for the nociceptive rhythm were, indeed, provided by the light-dark cycle and were not imposed by other possible cyclic factors in the animal quarters that may have generated a circadian rhythm in stress and, consequently, in the pain sensitivity. In the present study, attention was focused on the two time points in the photocycle (i.e., -2 hand +4 h), between which according to Bar-Or and Brown, the rats exhibited the maximal difference in nociceptive response. The present observation that nociceptive sensitivity was low at -2 h and high at +4 h was in agreement with that by Bar-Or and Brown (5). We have established and have reported separately that levels of urinary aMT6s, the principal metabolite of melatonin, were high at -2 hand low at +4 h in animals in the current study (22).

Functional pinealectomy, which in these rats (22) and in a previous study (19) was found to eliminate the diurnal rhythm of aMT6s level by abolishing the nighttime increase, also disrupted the normal pattern of diurnal rhythm in nociception. For example, the rhythm of pressure-elicited nociceptive response was effaced and that of heat-elicited response was reversed. While, in light of the observations by Bar-Or and Brown (5), the disappearance of the nociceptive rhythm was expected in functionally pinealectomized rats, its reversal was rather puzzling. Perhaps this difference in response to different stimuli may support the view that different nociceptive stimuli may act via different mechanisms (54). It has been reported that in humans, certain mechanical pains are associated with the activation of myelinated (Aδ) afferents, whereas unmyelinated (C) fiber discharges are accompanied by prolonged burning sensations (52). It has also been shown that noxious thermal stimuli may induce further increases in the sensitivity of polymodal nociceptors to mechanical stimuli (39). It should be pointed out in this connection that in their experiments, Bar-Or and Brown (5) used only thermal stimuli, whereas in the present study, both thermal and mechanical stimuli were applied. The possibility that these differences in stimuli

inherent in the experimental paradigm, may at least be partially responsible for the variation in the results, should not be ruled out.

A previous study in the mouse showed analgesic effects of melatonin in intact animals, i.e., using pharmacological administration of melatonin (30). In the present study it is clearly demonstrated that melatonin administration (replacement) in physiological doses can nullify the effect of light-induced functional pinealectomy. With melatonin replacement in functionally pinealectomized rats, the normal baseline rhythms of both pressure-elicited and heat-elicited analgesic responses displayed under 12:12 LD cycle, was reestablished. Although these results provide additional evidence to implicate melatonin in the modulation of diurnal rhythm of nociception, the mechanism of the analgesic action of this hormone has yet to be elucidated. Because studies in several vertebrate species including humans have reported many interactions between pineal and the opiate system (15,24,26,28,29,32,33,44,55), it is possible that an indoleaminergic-opioid system could be involved in modulating nociceptive sensitivity. It has been reported that melatonin administration or pinealectomy produces changes in the brain [Met^5]enkephalin content of rats (28) and hamsters (29). Some studies also support the contention that the pineal gland is able to synthesize, store, and release a specific pool of opioid peptides (7,37,50). Further, naloxone, an opioid antagonist, has been reported to block the analgesic effect of melatonin in mice (15,30).

Some studies have indicated that melatonin may have a link to benzodiazepine systems, which are also believed to be implicated in the mediation of nociceptive sensitivity. For example, melatonin and its brain metabolite (N-acetyl-5-methoxykynurenamine) have been shown to be potent endogenous ligands for the benzodiazepine binding site of the y-aminobutyric acid (GABA) receptor in rats (35). Further, pinealectomy has been shown to disrupt the circadian rhythms of benzodiazepine (1,27) and GABA binding (2) in the rat brain, with melatonin treatment having a counteracting effect. It has also been shown that melatonin increases brain GABA concentrations in the rat (4). In addition, it has been observed in mice that the analgesic effect of melatonin was blocked by the central-type benzodiazepine antagonist Ro 15-1788 and, conversely, was enhanced by agonist, diazepam (15). A role for benzodiazepine systems in the mediation of melatonin-induced nociception in rats, therefore, cannot be ruled out.

Serotonin (5-hydroxytryptamine) could be another suspect involved in mediating the analgesic effect of melatonin. Serotonin and many of its agonists and precursors are known to enhance morphine analgesia (8,11,34,49,51,53). It has also been demonstrated that intraperitoneal administration of melatonin could elevate brain serotonin concentration (3). In view of the existence of an interrelationship between melatonin and the central serotonergic system (3, 13) and the role that serotonergic neurons play in controlling opioid-mediated analgesia (31), Golombek et al. (15) suggested the existence of a possible link between the two mechanisms, implying that the effect of melatonin is mediated via a central serotonergic-opioid system. It may be pointed out, however, that some studies found no direct evidence for the analgesic action of serotonin (45) and some others indicated an antagonistic action (17). Assessing the effect of serotonergic agents on analgesia, Dennis and Melzack (11) remarked that the differences in the type of noxious stimulation and in the motor responses required in various pain tests are crucial in determining the observed pharmacological profile of pain modulation.

The reports that in certain vertebrates, melatonin may cause vasodilation (14,21), as was found in response to certain pain relief treatments such as acupuncture and transcutaneous nerve stimulation (23), may provide supporting evidence for the involvement of melatonin in analgesia. The effectiveness of acupuncture in analgesia has been correlated with the degree of cutaneous vasodilation (38).

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