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Pharmacological effects of melatonin treatment on both locomotor activity and brain serotonin release in rats

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Abstract: The effects of intraperitoneal administration of pharmacological doses of melatonin (60 mg/kg) on both locomotor activity and brain monoamine release were assessed in rats. The spontaneous levels of either horizontal motion, vertical motion, or total distance traveled were decreased following melatonin injection. On the other hand, the spontaneous levels of postural freezing increased after treatment. External heat exposure (36°C) produced increases in locomotion (including horizontal motion, vertical motion, and total distance traveled) as well as decreases of postural freezing in rats. The heat-induced increases of horizontal motion and total distance traveled as well as decreases of postural freezing were attenuated by melatonin treatment. In addition, cold exposure (4°C) produced increases of vertical motion as well as decreases of postural freezing. Again, the cold-induced behavioral responses were attenuated by melatonin treatment. Biochemical data revealed that the serum levels of melatonin were decreased by both heat and cold exposure in rats. Furthermore, voltammetric data revealed that intraperitoneal administration of melatonin (60 mg/kg) decreased serotonin, but not the dopamine, release in the hypothalamus, the corpus striatum or nucleus accumbens of rat brain. Neither the locomotor activity responses to thermal stress nor brain monoamine release was affected by a smaller dose of melatonin (30 mg/kg, i.p.). The results suggest that systemic administration of melatonin, at pharmacological doses, inhibits brain serotonin release and results in a reduction in both the spontaneous locomotion and the thermal stress-induced locomotor activity responses in rats.

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Key words: melatonin—serotonin—locomotion—cold—heat—brain

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Introduction

Melatonin, which is synthesized in the pineal gland, may play an important role in modulation of locomotor activity [Data and King, 1980; Rodriguez et al., 1984]. Systemic or intracerebral administration of melatonin to animals decreased spontaneous locomotor activity [Kovacs et al., 1974; Golus and King, 1981; Sugden 1983; Gaffori and Van Ree, 1985]. In addition, it was found that administration of serotonin antagonists (such as methysergide and cyproheptadine) into the brain resulted in behavioral changes similar to those found after treatment with melatonin [Gaffori and Van Ree, 1985]. Furthermore, microinjection of serotonin and various antidepressant drugs into the brain completely inhibited the melatonin-induced behavioral responses. These observations suggest that melatonin interacts with brain serotoninergic mechanisms to influence locomotor activity. However, evidence that melatonin directly affects brain serotonin release or turnover in rats is lacking. It is not known whether the behavioral responses to thermal stress are modulated by melatonin treatment.

In order to deal with the above-mentioned questions, experiments were carried out to assess the effects of intraperitoneal injection of melatonin on

both the locomotor activity responses to external heat or cold exposure and monoamine release in different brain regions using carbon fiber electrodes combined with differential pulse amperometry [Chuang et al., 1993; Lin et al., 1993; Yang and Lin, 1993]. Additionally, the effects of cold or heat exposure on serum levels of melatonin were also assessed.

Materials and methods

Male Sprague-Dawley rats weighing between 250 and 300 g were used in the present experiments. Upon receipt from the supplier (Animal Resource Center, National Cheng Kung University Medical College, Tainan City, Taiwan, ROC), the animals were housed in pairs in a temperature-regulated (22 ± 1°C) room on a 12:12 light:dark cycle with food and water ad libitum. The light was turned on at 0600 and turned off at 1800.

The behavioral apparatus used were four activity chambers equipped with an infrared light matrix system as detailed elsewhere [Young et al., 1993]. The following locomotor activities were measured using this system: (1) horizontal fine movement time (HFMT; the time elapsed for a horizontal displacement of less than 1.6 cm), (2) horizontal gross movement time (HGMT; the time elapsed for a horizontal displacement of greater than 1.6 cm), (3) vertical movement time (VMT; the time elapsed for vertical displacement in which one or more infrared light beams on the Z-axis were blocked), (4) posture freezing time (FT; the time elapsed when the animal did not have HFMT, HGMT, or VMT); and (5) total distance traveled (TDT; the total sum of all HGMT). An animal was placed in the center of the open field and was allowed to habituate to the open field for 60 min 1 week before testing. Each rat was tested only once. While the rat was being injected with drugs, the open field was washed. The rat was then returned to the open field for observation immediately after injection. All behavioral testings were done in the light phase of the diurnal cycle during the period of 1000–1400.

Three groups of animals were acclimatized at each selected ambient temperature (T_a: 4°C, 22°C and 36°C) for at least 60 min before they were subjected to decapitation at 1400. Serum melatonin levels were measured using a melatonin RIA kit (CIDtech Research Inc.) and a method modified from that described by Brown et al. [1985]. One-half ml of serum was extracted with dichoromethane, and the phases separated by centrifugation at 3,000*g*. The aqueous layer was aspirated and the tubes placed in dry ice for 40 min to freeze the remaining water and interphase to the side of the tube. The organic phase was then decanted and evaporated to dryness under nitrogen in a 37°C water bath. The residue was reconstituted in the buffer (pH 7.5, 0.05 M phosphate buffer containing 0.1% gelatin) and was added by 2,000 cpm of 3H-melatonin and CIDtech rabbit anti-melatonin serum for a final volume of 0.65 ml. Following at least 19 hr incubation at 4°C, globulins and bound ligand were precipitated by adding an equal volume of saturated ammonium sulfate containing calcium sulphate suspension. Following centrifugation the supernatant was decanted, and the residue redissolved in 0.55 ml of deionized water of which 0.5 ml was pipetted into scintillation vials and counted. Within assay variability was 7.6% for a sample of 24.4 pg/ml and between assay variability was 9.4%. The minimum level of detectability for this method was 5 pg/ml serum for melatonin.

A single carbon fiber (12 μm in diameter, AVCO, Lowell, MA, USA) was inserted into pulled glass micropipette (20–25 mm in length). The pipette tip was cut, and then the carbon fiber was pushed out of the pipette tip. Electrical contact with the fiber was made using silver paste. The tip and blunt end of the pipette were sealed with cyanoacrylate adhesive (super glue). The entire surface of a pyrolytic carbon fiber was 12-μm thick and 500-μm long. To improve the sensitivity and the selectivity of carbon fiber electrodes for monoamines, the electrodes were electrically pretreated using a modified protocol of Gonon et al. [1981]. The tip of carbon fiber electrodes were immersed into a nafion drop (10 μl of 5% solution; Aldrich Chemical Company, Inc., Milwaubel, WI, U.S.A.) four times. The nafion-coated electrodes were then dried at 60°C for 20 sec and used immediately for measuring monoamine release [Crespi et al., 1988; Chuang et al., 1993; Lin et al., 1993; Yang and Lin, 1993]. Differential pulse amperometry was performed in vitro and in vivo with a Biopulse (Solea TACUSSEL Co., France). To determine the selectivity of these nafion-coated electrodes for monoamine, a ratio of the sensitivity of serotonin/5-hydroxyindoleacetic acid, serotonin/dopamine, serotonin/norepinephrine, or serotonin/DOPAC was determined. The electrodes used for assessment of serotonin were ordinarily 200 times more sensitive to serotonin than 5-hydroxyindoleacetic acid and were completely nonsensitive to dopamine. Similarly, the electrodes used for assessment of dopamine were ordinarily 200 times more sensitive to dopamine than DOPAC and were completely nonsensitive to serotonin.

Animals were anesthetized with sodium pentobarbital (6 mg/100 g, i.p.), held in a stereotaxic frame and implanted with a nafion-coated electrode

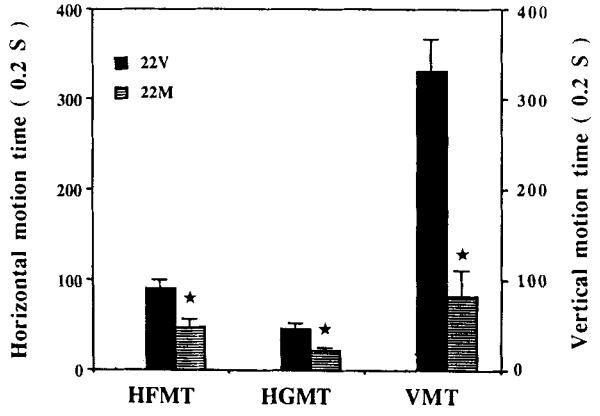

Fig. 1. The mean and standard error values (n = 8 for each group) of horizontal fine motion time (HFHT), horizontal gross motion time (HGMT), or vertical motion time (VMT) obtained from vehicle-treated rats (22V) and melatonin-treated rats (22M) kept at an ambient temperature (T_a) of 22°C during a 60-min observation period. *Significantly different from corresponding control values (vehicle-treated group), $P < 0.05$ (One-way ANOVA).

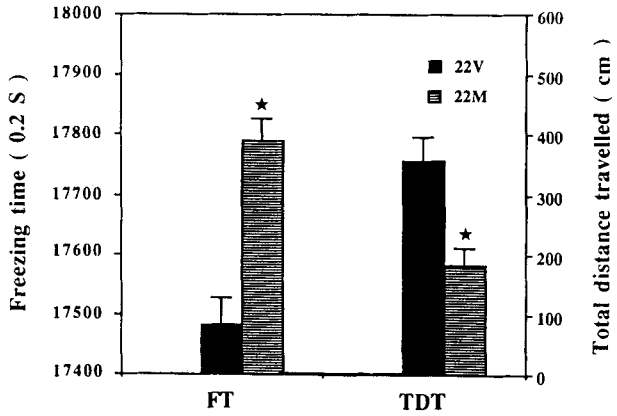

Fig. 2. The mean and standard error values (n = 8 for each group) of postural freezing time (FT) and total distance traveled (TDT) obtained from vehicle-treated (22V) and melatonin-treated rats (22M) kept at a T_a of 22°C. *Significantly different from corresponding control values (vehicle-treated group), $P < 0.05$ (One-way ANOVA).

in either the anterior hypothalamus, the corpus striatum, or the nucleus accumbens using the coordinates of Paxinos and Watson [1982]. Auxiliary (silver wire) and reference (Ag/AgCl) electrodes were placed on the dura surface of the parietal skull. They were anchored with fast-drying dental cement to the cranial surface. The animals were subjected to experimentation 2 or 3 days after electrode placement. Differential pulse voltammograms were recorded automatically every 2 sec. At the end of each experiment, an electrolytic lesion was performed by applying a continuous potential (+5 V) for 3 sec to the carbon fiber electrode. The current passing through the electrode was about 0.4 nA. The brain was dissected, frozen, and kept at −20°C. The brain was cut into 20-μm coronal slices. Every third section was collected for Nissl's staining.

Results

Effects of melatonin on spontaneous locomotor activity

Two groups of animals (n = 8 for each group) were acclimatized to the open field at an ambient temperature (T_a) of 22°C and their locomotor activity responses to i.p. injection of melatonin (60 mg/kg) are summarized in Figures 1 and 2. Compared with those of the vehicle treated rats, the melatonin-treated rats had a lower value for horizontal fine motion time, horizontal gross motion time, vertical motion time, or total distance traveled, but a higher value of postural freezing time.

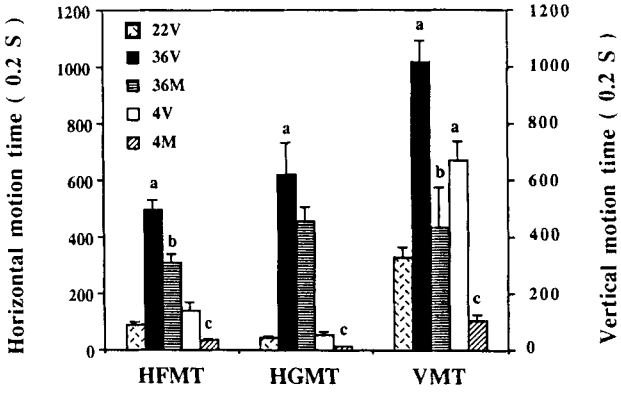

Fig. 3. The mean and standard error values (n = 8 for each group) of HFMT, HGMT, and VMT obtained from vehicle-treated rats kept at an ambient temperature (T_a) of 22°C (22V), vehicle-treated rats kept at a T_a of 36°C (36V), melatonin-treated rats kept at a T_a of 36°C (36M), vehicle-treated rats kept at a T_a of 4°C (4V), and melatonin-treated rats kept at a T_a of 4°C (4M) during a 60-min observation period. [a]Significantly different from the control values (22V group), $P < 0.05$ (One-way ANOVA). [b]Significantly different from the control values (36V group), $P < 0.05$ (One-way ANOVA). [c]Significantly different from the control values (4V group), $P < 0.05$ (One-way ANOVA).

Effects of melatonin on locomotor activity responses to thermal stress

Five groups of animals (n = 8 for each group) were exposed to a selected T_a of either 22°C, 36°C, or 4°C for 60 min, and their locomotor activity responses to melatonin or vehicle injection are summarized in Figures 3 and 4. Compared with those of the vehicle-treated rats kept at T_a = 22°C (22 SV), animals treated with vehicle kept at T_a = 36°C had

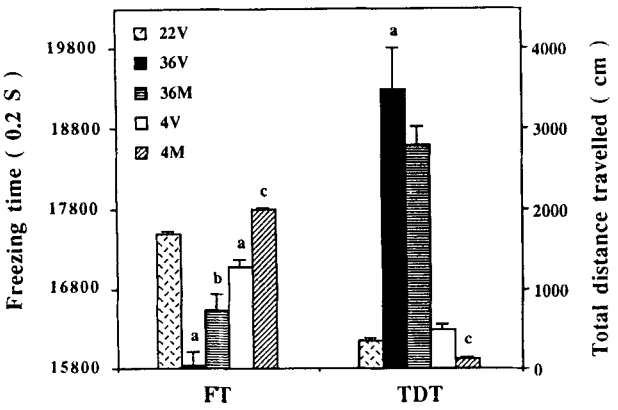

Fig. 4. The mean and standard error values (n = 8 for each group) of FT and TDT obtained from vehicle-treated rats at an ambient temperature (T_a) of 22°C (22V), vehicle-treated rats at T_a = 36°C (36V), melatonin-treated rats at T_a = 36°C (36M), vehicle-treated rats at T_a = 4°C (4V), and melatonin-treated rats at T_a = 4°C (4M) during a 60-min observation period. [a]Significantly different from the control values (22V group), $P < 0.05$ (One-way ANOVA). [b]Significantly different from the control values (36V group), $P < 0.05$ (One-way ANOVA). [c]Significantly different from the control values (4V group), $P < 0.05$ (One-way ANOVA).

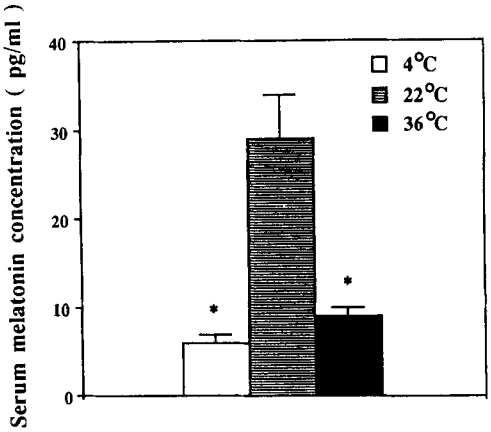

Fig. 5. The mean and standard error values (n = 8 for each group) of serum melatonin obtained from rats exposed to T_a = 4°C, rats exposed to T_a = 22°C, and rats exposed to T_a = 36°C for 90 min. *Significantly different from the control value (T_a = 22°C group), $P < 0.05$ (One-way ANOVA).

a higher value of either horizontal fine motion time, horizontal gross motion time, vertical motion time, or total distance traveled (Fig. 3), but a lower value of postural freezing time (Fig. 4). Both the increases of horizontal fine motion time and vertical motion time and the decreases of postural freezing time produced by heat exposure were reduced by melatonin administration (60 mg/kg, i.p.). Compared with those of the vehicle-treated rats kept at T_a = 22°C, the vehicle-treated rats kept at T_a = 4°C had a higher value of vertical motion time, but a lower value of postural freezing time (Figs. 3, 4). Again, the cold-induced increases of vertical motion time as well as decreases of postural freezing time were reduced by melatonin treatment.

Effects of cold or heat exposure on serum melatonin levels

Three groups of animals (n = 8 for each group) were acclimatized to a selected T_a of either 22°C, 36°C, or 4°C for 60 min, and melatonin levels in the serum were measured (Fig. 5). Compared with those of the T_a = 22°C group, both the T_a = 36°C group and the T_a = 4°C group had a lower value of serum melatonin.

Effects of melatonin treatment on brain monoamine release

Table 1 summarizes the effects of melatonin injection on monoamine release in different brain regions using the previously implanted carbon fiber electrodes combined with differential pulse amperometry. It can be seen from the Table 1 that i.p. injection of melatonin (60 mg/kg), but not the vehicle, decreased the serotonin release from the hypothalamus, the corpus striatum, and the nucleus accumbens. On the other hand, melatonin administration produced no significant changes in catecholamines release in either the hypothalamus, the corpus striatum, or the nucleus accumbens. A typical example showing the time course changes of striatal serotonin and catecholamines release in response to melatonin treatment is depicted in Figure 6.

Neither the locomotor activity responses to thermal stress nor brain monoamine release was affected by a small dose of melatonin (30 mg/kg, i.p.; the data are not shown).

Discussion

Previous results showed that the serotonin metabolism in the nucleus accumbens may relate to melatonin-induced locomotor activity responses in rats [Jones et al., 1981; Gaffori and Van Ree, 1985]. Administration of melatonin into the nucleus accumbens produced a decrease of locomotor activity. The action of melatonin was mediated by the brain serotonin system, because the melatonin-induced behavioral changes were antagonized by local pretreatment of nucleus accumbens with serotonin or various antidepressant drugs, but not with dopaminergic receptor antagonists such as haloperidol and sulpiride. Injection of serotonin antagonists (such as methysergide and cyproheptadine) into the nucleus accumbens resulted in behavioral responses similar to those found after treatment with melatonin. The

TABLE 1. *Effect of melatonin on monoamine release in different brain regions of rats*[a]

Treatment	Changes in serotonin release (ΔnM)[b]			Changes in dopamine release (ΔnM)[b]		
	Hypothalamus	Striatum	Nucleus accumbens	Hypothalamus	Striatum	Nucleus accumbens
Control vehicle, i.p.[c]	−2 ± 1(6)	−3 ± 2(6)	−5 ± 2(6)	+3 ± 2(8)	−4 ± 2(4)	+4 ± 2(4)
Melatonin 60 mg/Kg, i.p.	−272 ± 38(6)*	−178 ± 34(8)*	−165 ± 43(4)*	+8 ± 3(8)	+7 ± 4(4)	+5 ± 3(4)

[a]The values are expressed as means ± SEM, followed by number of rats in parentheses.
[b] The delta values denote the difference between the control values before and 60 min after the start of injection.
[c]The control values for serotonin release in the hypothalamus, the striatum, and the nucleus accumbens, respectively, are 612 ± 52 nM, 573 ± 49 nM, and 551 ± 44 nM. The control values for dopamine release in the hypothalamus, the striatum, and the nucleus accumbens, respectively, are 213 ± 22 nM, 256 ± 25 nM, and 234 ± 21 nM.
*Significantly different from corresponding control values (10% alcohol injection of each group), $P < 0.05$ (One-way ANOVA).

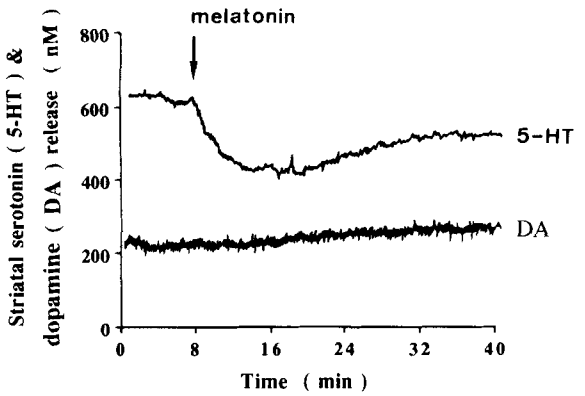

Fig. 6. The time course change of striatal serotonin (5-HT) and catecholamine (CA) release produced by i.p. injection of melatonin (60 mg/kg) in two rats (one for 5-HT release recording and the other for CA release recording).

results suggest that brain serotonin, rather than dopamine, is involved in the behavioral changes seen after treatment with melatonin. The present results demonstrated that melatonin, when administered systemically, produced decreases in locomotor activity (including horizontal motion, vertical motion and total distance traveled) as well as increases in postural freezing in rats. The voltammetric data also revealed that the serotonin, but not dopamine, release from the hypothalamus, the corpus striatum, and the nucleus accumbens were decreased after systemic administration of melatonin. These observations tend to indicate that systemic administration of melatonin inhibits brain serotonin release and results in a decrease in spontaneous locomotor activity in rats.

Some inconsistent results, however, have also been reported [Anton-Tay et al., 1968; Carman et al., 1976]. For example, intraperitoneal administration of melatonin to rats caused an increase in the midbrain and the hypothalamic serotonin concentration [Anton-Tay et al., 1968]. Carman et al. [1976] showed an increase in 5-hydroxyindoleacetic acid level in the cerebrospinal fluid following melatonin injection in depressed patients. Therefore, additional experimental data are needed for a more conclusive conclusion concerning the effects of melatonin treatment on serotonin metabolism in brain.

In the current studies, external heat exposure (36°C) increased locomotor activity (including horizontal motion, vertical motion, and total distance traveled) as well as decreasing postural freezing in rats. The heat-induced increases in horizontal fine motion and vertical motion as well as decreases of postural freezing were reduced by melatonin treatment. The heat-induced increases of horizontal gross motion and total distance traveled were not affected by melatonin. The present results also showed that cold exposure (4°C) increased vertical motion as well as decreased postural freezing in rats. Again, the cold-induced behavioral responses were attenuated by melatonin treatment. The results reported here provide new evidence that systemic administration of melatonin inhibits a number of locomotor activity responses to external heat or cold exposure. In addition, both the present and the previous [Welker and Vollrath, 1984; Tannenbaum et al., 1988; Troiani et al., 1988] results showed that either cold or heat exposure decreased the serum melatonin levels in rats. Thus, it appears that the locomotor activity responses to heat or cold are related to melatonin metabolism in rats. As discussed above, systemic administration of melatonin decreased serotonin release in different brain regions. Our recent findings [Lin et al., unpublished data] also shown that brain serotonin release was elevated in rats placed in cold or heat. Therefore, it is possible that melatonin, when administered systemically, inhibits brain serotonin release, which results in a reduction in the locomotor activity responses to heat or cold.

In summary, the present results showed that systemic administration of pharmacological doses of melatonin to rats reduces horizontal motion, vertical motion, and total distance traveled, while it increases postural freezing. External heat exposure

produced the opposite behavioral effects in rats. External cold exposure increased vertical motion and decreased postural freezing. Both heat-induced increases of horizontal gross motion and total distance traveled, the cold-induced vertical motion, and decreases of postural freezing induced by heat or cold exposure were attenuated by melatonin treatment. Biochemical data showed that serum melatonin levels were decreased by both heat and cold exposure. Voltammetric data also revealed that melatonin treatment decreased serotonin release from the hypothalamus, the corpus striatum, and the nucleus accumbens of rat brain. The results indicate that systemic administration of melatonin inhibits brain serotonin release and results in a reduction in both the spontaneous locomotion and the thermal stress-induced locomotor activity responses in rats.

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