Effects of Maternal-Melatonin Treatment on Open-field Behaviors and Hypertensive Phenotype in Spontaneously Hypertensive rats' (SHR) Offspring

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Abstract: Effects of maternal-melatonin treatment in spontaneously hypertensive rats (SHR) were investigated in their offspring. Pregnant SHR were given drinking water with/without melatonin (20 µg melatonin/ml tap water) during pregnancy and the lactation period. Maternal-melatonin treatment did not cause changes in body weights during 7 to 27 weeks. Melatonin administration up to weaning period via mother caused a decrease in systolic blood pressure (BP) during 11 to 27 weeks in their offspring compared with those of control group. Open-field behaviors in the offspring were observed at 24 weeks age. Both the control and treatment groups had ratios of central and peripheral locomotion of 30% and 70%, respectively. The treatment group exhibited less total locomotor activity and rearing than the control group did, whereas more latency was exhibited in the treatment group compared with that of the control group. These findings suggest that maternal-melatonin administration may modify open-field behaviors as well as the hypertensive phenotype in their progeny.

Key words: behavior, blood pressure, melatonin, open-field, SHR

Introduction

Spontaneously hypertensive rats (SHR) have been selectively inbred for the hypertensive trait, a feature which makes it the most widely used animal model of human essential hypertension [17, 25]. SHR are also endowed with a behavioral abnormality [11] and are thus suggested to be a natural animal model of attention deficit hyperactivity disorder (ADHD) [21]. It is well known that SHR habituate poorly and show high

activity scores in a novel environment [11, 12]. In open-field of a novel environment, SHR showed higher locomotor activity and exploratory rearing behavior [22]. Such behavioral abnormality was suggested to play a role in the development of hypertension in SHR [22].

It has been reported that the idiosyncratic behavior of hypertensive mothers contributes to the elevated blood pressure (BP) of their offspring [3]. Hypertensive rats reared by normotensive foster mothers have

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significant reductions in BP in adulthood [2, 16]. Also, SHR fostered by either Wistar Kyoto or Sprague-Dawley dams displayed a greatly reduced blood pressure (BP) compared with SHR reared by their natural mothers [6]. This indicates that the development of hypertension in SHR is dependent on the hypertensive maternal environment during the neonatal period as well as on the genetic predisposition. Thus, the neonatal period for maternal care seems to be a critical period for triggering the full expression of the hypertensive phenotype in SHR, since rat offspring have a brain growth spurt after birth [7].

Melatonin, a neuro-endocrine hormone secreted by the pineal gland, is transported via the blood to all tissues where it enters cells [18]. It may pass from the mother to the fetus and affect its developing nervous system [19]. Melatonin effects on rodent behavior are inhibitory, such as sedative, analgesic, and anticonvulsant effects and anxiolytic activities, and it also has direct effects on circadian rhythmic activity (e.g., entrainment, resynchronization) [8]. Thus, it is possible that such melatonin-induced behavioral activities may affect the idiosyncratic behavior of hypertensive mothers contributing to elevated BP in their offspring, resulting in modification of the hypertensive phenotype or a behavioral abnormality.

To evaluate this possibility, pregnant SHR were treated with melatonin up to the weaning period. We ascertained whether maternal treatment with melatonin affected open-field behaviors as an indicator of behavioral abnormality in their offspring. Systolic BP was also monitored at regular intervals.

Materials and Methods

Animals

Ten weeks old spontaneously hypertensive rats (SHR) (body weight ranging from 160 to 180 g) were obtained from a breeding colony maintained at Dept. of Lab. Animals of the Asan Medical Center (Seoul, Korea). They were housed in plastic rat cages with *ad libitum* access to feed and water. They were kept in a temperature-controlled room (23 ± 2 C) with a 12-hr light-dark cycle (light, 0600–1800 hr). Twenty-four females were mated with 8 male rats. They were checked every morning for the presence of a vaginal plug and the 7 females showing a vaginal plug were

selected for this study: 4 females for the control group and 3 females for the treatment group. When pregnancy was assured, the females were isolated from the males and kept in separate cages until delivery. On the day after delivery, the number of pups was adjusted to seven or eight (male three to four and female four if possible) in each litter. The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, Asan Institute for Life Sciences and University of Ulsan.

Maternal treatment of melatonin

Melatonin was solubilized in ethanol, and mixed in drinking water (20 μ g melatonin/ml tap water, 0.05% ethanol). The seven females showing a vaginal plug were administered drinking water with/without melatonin during pregnancy and the lactation period. After weaning, both groups were given tap water without melatonin, and only male offspring were raised for the measurements of systolic BP and open-field behavior in the study.

Blood pressure monitoring

Systolic BP was monitored at 11, 15, 19, 23, and 27 weeks of age in male offspring. Systolic BP measurements were made with pre-heating using the tail-cuff method. The pulse of the rat tail artery was detected photoplethysmographically by a blood pressure monitor (MK-1000, Muromachi Kikai Co., Ltd. Tokyo, Japan). Systolic BP was determined at the pulse appearing point. Averages of systolic BP were calculated from four measurements.

Open-field test

At 24 weeks age, male offspring were observed in an open-field apparatus. The open-field (OF) apparatus was made of white-painted plywood. The floor of the apparatus measured 100×100 cm and was divided into 25 evenly spaced squares defined by black lines made of adhesive tape that were stuck on the floor. The floor was surrounded by a 50-cm high, opaque white wall. The open-field apparatus was illuminated by a 60-W bulb placed 1.5 m above the apparatus. A charge-coupled device (CCD) camera was mounted directly above the open-field apparatus. A fan motor in the room provided constant background noise. Each rat was moved from its home cage to the center square of

the open field, and an opaque square box (20 × 20 × 20 cm) was then placed over the rat.

The open-field test was conducted by a general procedure described in detail elsewhere [1, 15, 23], except as noted below. Briefly, after 20 sec, the box was gently removed, and the behavior of the rat was observed for the following 3 min. The behavior was also recorded with a digital video recorder (Hancorn Co. Ltd., Korea) for counting the number of locomotor activities. A locomotion was defined as a movement in which both the hind paws entered a new square. The 25 squares were classified as either peripheral (the sixteen squares adjacent to the wall) or central (the nine remaining squares in the center). The scores were also classified according to whether the square entered was peripheral or central. During a 3-min period, center square latency (latency) (the time until the rat first entered into the other squares in the open-field) and rearing (number of times the rat stood on hind legs) were also observed. Before each trial the floor and wall were cleaned with 70% alcohol followed by wet cotton. All the trials were done between 1300 and 1500 hr.

Statistics

Means of activity scores such as locomotion, rearing, and latency in open field were calculated, and the differences between the treatment and control groups were analyzed by Student's *t*-test. For body weight and systolic BP, data were analyzed by repeated measures analysis of variance (ANOVA) with maternal-melatonin treatment as a between-subject factor (drinking water with/without melatonin). All statistical calculations were performed by the SPSS statistical package (version 10.0, SPSS Inc. 1999).

Results

Body weights in male offspring

During pregnancy and lactation, none of the mothers in either of the groups died, nor did offspring show any abnormal appearance. Table 1 shows changes in body weights of offspring during 7 to 27 weeks. Repeated measures ANOVA on body weight data showed that body weights in the treatment group were not changed compared with those of the control group [$F(1, 24)=0.139, p=0.712$].

Table 1. Body weight changes in male offspring

	Body weight (g)	
	Control	Treatment
7 w	172.2 ± 12.9	173.7 ± 13.1
11 w	276.8 ± 15.2	278.3 ± 15.3
15 w	318.9 ± 14.6	306.5 ± 18.8
19 w	348.6 ± 14.8	348.0 ± 17.6
23 w	356.5 ± 21.4	357.9 ± 18.6
27 w	381.7 ± 17.2	377.3 ± 17.9

Values are mean ± SD. There was no significant difference between the control and treatment groups [$F(1, 24)=0.139, p=0.712$].

Fig. 1. Change in systolic blood pressure. Mean and SD are shown ($n=11-15$). Repeated measures ANOVA on systolic BP data showed that systolic BP in the treatment group were significantly lowered compared with those of control group [$F(1, 24)=13.226, p=0.001$].

BP monitoring

We monitored changes of systolic BP at an interval of 4 weeks in male offspring. As shown in Fig. 1, systolic BP gradually increased with time in the control group. Repeated measures ANOVA on systolic BP data indicated that maternal-melatonin treatment caused a decrease in systolic BP during 11 to 27 weeks in the treatment group [$F(1, 24)=13.226, p=0.001$].

Open field test

Open-field behaviors in male offspring were observed at 24 weeks of age. Correlation coefficients between total locomotion and rearing or between rearing and

Table 2. Pearson's correlation coefficients for open-field behaviors of both groups

	Total locomotion	Rearing	Latency
Total locomotion	1.000	0.613**	-0.354
Rearing	0.613**	1.000	-0.498**
Latency	-0.354	-0.498**	1.000

Each correlation coefficient was calculated by data from both groups. Total locomotion was number of the squares traversed for 180 sec in open-field. During a 3-min period, center square latency (the time until the rat first entered into the other squares in open-field) and rearing (number of times the rat stood on hind legs) were also observed. ** $p < 0.01$.

Fig. 2. Central and peripheral locomotion in open field. Each bar represents the mean $\pm$ SD. It shows relative percentage of locomotion in the central and peripheral area. There was no significant difference between the control and treatment groups. Locomotion was defined as No. of times both hindpaws entered a new square. Percentage of central or peripheral locomotion was defined as follows: $100 \times (\text{No. of squares traversed in the center or peripheral area}) / (\text{No. of total squares traversed})$.

latency are shown in Table 2. Total locomotion was positively related with rearing, whereas rearing was negatively related with latency. Both the control and treatment groups had ratios of central and peripheral locomotion of 30% and 70%, respectively (Fig. 2). As shown in Fig. 3, the treatment group exhibited less total locomotor activity and rearing than the control group did, whereas it exhibited more latency than the control group did.

Discussion

Regarding melatonin toxicity in pregnant rats, maternal toxicity NOAEL was 100 mg/kg/day, while developmental toxicity NOAEL was ≥ 200 mg/kg/day

Fig. 3. Open-field behaviors in offspring. Each bar represents the mean $\pm$ SD. Upper figure (a) shows total locomotion as number of the squares traversed for 180 sec in open-field. Middle figure (b) shows rearing as number of times the rat stood on hind legs. Lower figure (c) shows center square latency as the time (seconds) until the rat placed in center square first entered into the other squares in open-field. * $p < 0.05$, ** $p < 0.01$.

at all any of the endpoints related to embryo and fetal growth, viability, or morphological development [13]. In the present study, we administered drinking water containing melatonin to pregnant SHR. The estimated dose of melatonin, as calculated from water consumption (about 40 ml per day), was 4 to 5 mg/kg/day, at least 40 times less than the developmental toxicity NOAEL mentioned above. None of the mothers in either of the two groups died, nor did offspring show any abnormal appearance. Body weight gains in the treatment group were not changed compared with those

of the control group (Table 1).

The hypotensive effect of melatonin has been observed in patients with essential hypertensive symptoms after intranasal administration for one week [4]. In rats, an antihypertensive action of melatonin was reported by Kawashima *et al.* [14]; melatonin administration with a dose of 6 mg/rat per day for 5 days produced a significant decrease in BP in adult male SHR. As shown in Fig. 1, systolic BP gradually increased with time in the control group. Repeated measures ANOVA on systolic BP data indicated that maternal-melatonin treatment caused a decrease in systolic BP during 11 to 27 weeks of age in the treatment group [$F(1, 24)=13.226, p=0.001$]. This result reveals the very interesting possibility that the level of BP in adulthood may be regulated by maternal-melatonin administration during pregnancy and the lactation period. It is likely that the decrease in systolic BP is associated with maternal care, since the maternal environment has been shown to play an important role in the development of high BP in genetically hypertensive rats [2, 16]. Also, SHR fostered by either Wistar Kyoto or Sprague-Dawley dams displayed a greatly reduced BP compared with SHR reared by their natural mothers [6]. Melatonin effects on rodent behavior are inhibitory, such as sedative, analgesic, and anticonvulsant effects and anxiolytic activities, and it also has direct effects on circadian rhythmic activity (e.g., entrainment, resynchronization) [8]. Thus, the present finding of a decrease in systolic BP can also be explained by a melatonin-induced alteration in the maternal environment, although the exact effect of melatonin on the maternal environment remains to be elucidated.

SHR have been suggested to exhibit some of the characteristics of human hyperactivity disorder and to be a natural animal model of ADHD [21]. The behavioral symptoms are problems with sustained attention (inattentiveness), overactivity and impulsiveness, which is associated with altered dopamine function [20]. ADHD is one of the most common childhood disabilities and affects between 3 and 5% of grade-school children [20]. In open-field of a novel environment, SHR also showed higher locomotor activity and exploratory rearing behavior [22]. Interestingly, SHR showed dopamine hypofunction like that of ADHD [21, 24]. The pharmacological effect of melatonin may involve modulation of the central dopaminergic functions

[10]. However, the effect of melatonin on locomotion has not yet been clarified; melatonin treatment caused a significant decrease in locomotor activity in hamsters [9], whereas melatonin administration increased open-field ambulatory behavior in rats [5]. Anyway, these studies do not directly explain the present finding of alteration in open-field behavior.

In the present study, both the control and treatment groups showed ratios of central and peripheral locomotion of 30% and 70%, respectively (Fig. 2). The treatment group exhibited less total locomotor activity and rearing than the control group did, whereas it exhibited more latency than the control group did (Fig. 3). Very little is known about the effect of maternal-melatonin administration on open-field behaviors and BP in their progeny. It has been reported that the development of hypertension in SHR may be associated with a behavioral abnormality [22]. However, the idiosyncratic behavior of the hypertensive mothers may contribute to the elevated blood pressure of their offspring [3]. Taken together, it is possible that the maternal-melatonin administration during pregnancy and lactation period may affect open-field behavior as well as the hypertensive phenotype in their progeny. Further study is required to clarify the exact mechanism of melatonin on alteration of open-field behaviors in the offspring of melatonin-treated pregnant SHR.

The present study provides evidence that melatonin is a modifying factor on behavioral abnormality and hypertensive phenotype in SHR, an animal model of ADHD. It may also suggest that the pregnancy and lactation period may be critical for forming behavioral and hypertensive characteristics in adulthood in SHR, since rat offspring have a brain growth spurt after birth [7]. Such modifications in progeny might contribute to understanding of ADHD for more focused approaches to interventions.

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