Desynchronosis and Erosive and ulcerative Lesions of the Stomach in Rats Active or Passive in the Open-Field Test: Effect of Exogenous Melatonin

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 3, pp. 283-286, March, 2002
Original article submitted November 19, 2002

We studied the effect of a phase shift in circadian rhythms (desynchronosis) on the development of erosive and ulcerative lesions in the gastric mucosa in male Wistar rats with different behavioral activity in the open-field test. The animals kept under conditions of natural or shifted light-dark cycle were untreated or intraperitoneally received physiological saline (1 ml) and melatonin (1 or 2 mg/kg). Desynchronosis induced gastric ulcers in active rats not receiving injections or intraperitoneally injected with physiological saline. No gastric ulcers were found in passive animals kept under shifted light-dark cycle. Melatonin induced gastric ulcers in passive animals kept under natural light-dark cycle. Gastric ulcers were not found in active rats subjected to desynchronosis and receiving melatonin. Our results indicate that treatment with melatonin for the correction of changes induced by shifts in the light-dark cycle should be performed taking into account individual behavioral characteristics.

Key Words: *desynchronosis; erosive and ulcerative lesions of the stomach; active and passive rats; melatonin*

Shifts in biological rhythms of the organism (desynchronosis) are an urgent medical and biological problem. Normal biological rhythms can be impaired by various factors. The rhythms are upset by shift work and by rapid travel into different time zones. Shifts in biological rhythms suppress general activity, impair mental functions, and cause sleep-and-awaking disorders. These changes contribute to the development of desynchronosis and various internal diseases, including erosive and ulcerative lesions of the gastric mucosa [7].

Published data show that the pineal gland is involved in the stress response [6]. Previous experiments revealed the relationship between phasic changes in the reaction to stress and activity of the pineal gland. It was hypothesized that the pineal gland secretes biologically active substances involved in the stress reaction and shift in biological rhythms [1]. One of these compounds is melatonin predominantly produced by pinealocytes. Secretion of this hormone undergoes diurnal variations and depends on the exogenous light-dark cycle [10]. A large body of evidence indicates that melatonin can be used for the correction of shifts in normal diurnal rhythms induced by various factors [8].

Rats of various strains have different resistance to emotional stress [3]. Moreover, the individual resistance to stress differs in animals of the same species. The open-field (OF) test is widely used to predict the individual resistance of rats to emotional stress.

Here we studied the effect of melatonin on gastric ulceration in rats with different activity in OF kept under conditions of shifted light-dark cycle.

MATERIALS AND METHODS

Experiments were performed on 172 male Wistar rats weighing 260.32±1.79 g. The animals were kept in cages (5 rats per cage) at 22-26°C under artificial light-dark cycle (12-h light and 12-h dim red light) and had

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0007-4888/03/1353-0244$25.00

free access to water and food. The animals were adapted to laboratory conditions for 5 days under natural light-dark cycle (8.00-20.00, light; 20.00-8.00, dark).

On day 6 rat behavior was studied in OF for 5 min. OF was an area (57×57 cm) surrounded by walls (43 cm in height) and illuminated with a 100 W lamp. The floor was divided into 16 central squares and 20 peripheral squares. There were 9 holes (2.5 cm in diameter) in the floor. The following parameters were recorded: the latency of the first movement and entrance into the center of OF, number of crossed squares (ambulations) and rearing postures in the peripheral and central zones, time of grooming (sec), and number of explored holes. The index of activity (IA) in OF was calculated as:

$$IA=(AP+AC)/(LP1+LC),$$

where AP and AC are the numbers of crossed peripheral and central squares, respectively; and LP1 and LC are the latencies of the first movement and entrance into the center of OF, respectively. Depending on activity in OF, the rats were divided into behaviorally active (n=62), passive (n=63), and intermediate subgroups (n=47, Table 1). Further experiments were performed on active and passive animals.

Over the next 10 days the rats were kept under conditions of the natural (8.00-20.00, light; 20.00-8.00, dark) or shifted light-dark cycle (8.00-20.00, dark; 20.00-8.00, light). The animals were intraperitoneally injected with 1 ml physiological saline or melatonin (daily doses 1 or 2 mg/kg in 1 ml physiological saline) or did not receive injections. Experimental procedures (feeding, cleaning of cages, and injections) were performed at 13.00 or 1.00. Each group included 4-5 animals.

On day 17 the rats were decapitated under urethane anesthesia (1 g/kg intraperitoneally). The stomach was incised by the greater curvature. The number of erosive and ulcerative lesions of the gastric mucosa was determined under a binocular microscope. The state of gastric mucosa was expressed in points: normal (0 points), single erosions (no more than 5, 1 point), multiple erosions (more than 6) or 1 ulcer (2 points), and 2 or more ulcers (3 points).

The results were analyzed by multifactor ANOVA (injection×light-dark cycle×time of treatment) followed by Fisher's least square difference (LSD) test. Between-group differences were evaluated by nonparametric Wilcoxon test. The data are presented as means and standard errors.

RESULTS

Erosive and ulcerative lesions of the gastric mucosa were found in behaviorally active rats kept under natural light-dark cycle and receiving physiological saline during the daytime. The degree of gastric lesions was 0.5±0.2 points (p<0.05 compared to rats not receiving injections). It was probably associated with individual characteristics of animals and breeding conditions. Moreover, Wistar rats are predisposed to gastric ulceration that can occur even under normal conditions [4]. Erosive and ulcerative lesions of the gastric mucosa were not revealed in animals receiving melatonin in doses of 1 or 2 mg/kg during the daytime and nighttime.

We did not found erosive and ulcerative lesions of the gastric mucosa in passive rats kept under natural light-dark cycle and not receiving injections or treated with physiological saline. Gastric ulceration occurred in passive animals kept under natural light-dark cycle and receiving melatonin in a dose of 1 mg/kg during the daytime and nighttime (0.33±0.15 and 0.40±0.24 points, respectively, p<0.05 compared to not injected rats). Nighttime treatment with melatonin in a dose of 2 mg/kg induced erosive and ulcerative lesions of the gastric mucosa in passive rats (0.33±0.12, p<0.05 compared to not injected rats).

This effect of melatonin could be related to several reasons. First, rats kept under natural light-dark cycle were not exposed to adverse environmental factors (*e.g.*, desynchronosis). And second, plasma concentration of endogenous melatonin during the nighttime is much higher than during the daytime [5]. Probably, administration of exogenous melatonin to control rats at night served as an ulcerogenic factor. These results agree with our previous experiments. Exogenous melatonin prevented hypertrophy of the adrenal glands and development of gastric ulcers in rats during

TABLE 1. Behavioral Characteristics of Rats in OF (M±m)

Parameter		Active rats (n=62)	Intermediate rats (n=47)	Passive rats (n=63)
Index of activity		2.63±0.23	0.88±0.05	0.29±0.02
Number	peripheral rearing postures	16.24±0.90	15.13±0.91	10.86±0.71
	central rearing	1.13±0.26	0.64±0.13	0.32±0.09
	explored objects	9.03±0.58	7.81±0.53	4.92±0.53
Grooming, sec		10.03±1.52	12.23±2.18	14.92±2.04

emotional stress [2]. However, treatment of control animals with melatonin was followed by gastric ulceration and hypertrophy of the adrenal glands (stress-like reaction).

The shift in the light-dark cycle led to pronounced gastric ulceration in active rats not receiving injections or treated with physiological saline at night (0.60±0.24 and 0.75±0.30 points, respectively, $p<0.05$ compared to the natural light-dark cycle). These changes were observed at 1.00, but not at 13.00. Therefore, in rats kept under natural or shifted light-dark cycle erosive and ulcerative lesions in the gastric mucosa were formed in the subjective daytime ($F=4.34$, d. f.=1, $p<0.04$). In the subjective nighttime gastric ulceration did not occur under conditions of natural and shifted light-dark cycles. Published data suggest that plasma melatonin concentration is minimal in the daytime and maximum at night. This hormone possesses antiulcer activity [9]. It can be hypothesized that gastric ulceration in rats subjected to desynchronosis or other adverse conditions does not occur in the periods coinciding with high plasma concentration of endogenous melatonin.

In active rats receiving melatonin in doses of 1 and 2 mg/kg during the daytime and at night the shift in the light-dark cycle was not followed by the development of erosive and ulcerative lesions in the gastric mucosa (compared to animals not receiving injections or treated with physiological saline). Thus, administration of melatonin prevents gastric ulceration in active rats kept under shifted light-dark cycle.

Erosive and ulcerative lesions of the gastric mucosa were not found in passive rats kept under shifted light-dark cycle.

These results suggest that the shift in the light-dark cycle induces erosive and ulcerative lesions in the gastric mucosa in active rats, but not in passive animals. Exogenous melatonin in various doses protected the gastric mucosa in active rats subjected to desynchronosis. Melatonin treatment induces gastric ulceration in passive animals kept under natural light-dark cycle. These data confirm high significance of individual approach to the prediction of organism's resistance to adverse factors. Treatment with melatonin should be performed taking into account individual behavioral characteristics.

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