EMOTIONAL DISTURBANCE IN TWO MODELS OF MELATONIN DEFICIENCY: A COMPARATIVE STUDY

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Abstract

In the present study, the aim was to compare the role of melatonin deficiency on emotional status in two different models, chronic constant light (CCL) and pinealectomy in male Wistar rats. While the rats with pinealectomy (Pin) showed impulsive behaviour (increased motor activity and lack of anxiety), CCL-rats demonstrated higher anxiety in the elevated plus-maze test (EPM). Slight differences in depressive-like behaviour, measured by the saccharine preference test (SPT) and the forced swimming test (FST), were also detected. The CCL-rats exhibited anhedonia only during the active (dark) phase of the light-dark cycle whereas rats with removed pineal gland showed depressive behaviour without diurnal variations. Immobility in the FST was increased in the two models of melatonin deficiency. Exposure to CCL and removal of the pineal gland abolished the circadian fluctuations in plasma melatonin levels. Both models of melatonin deficit exhibited higher plasma corticosterone levels during the light period and blunted diurnal variations of the hormone. Our findings suggest that models of melatonin deficiency recapitulate several neurobiological alterations associated with melancholic depression. Future studies are needed to elucidate the precise mechanism related to the model-specific difference in emotional status.

Key words: melatonin deficiency, emotional status, corticosterone, rat

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Introduction. All functions in the body are subject to rhythmic oscillations, which are determined from the chief biological clock by neural and humoral regulation [1]. These rhythms optimize the functions of cells and prevent or reduce the possibility of functional disorders. Endogenous biological rhythms can be changed by environmental factors called synchronizers. One of the most important synchronizers is the light in which control is exerted through retino-hypothalamic tract and hormones via the secretion of melatonin. The pineal gland is the main source of circulating plasma melatonin and its rhythmic secretion modulates the circadian dynamics of many physiological functions [1]. The gland receives neuronal projections from the main biological clock, the suprachiasmatic nucleus (SCN). This state determines the daily rhythm of hormonal synthesis, secretion, and synchronization of physiological parameters with light-dark cycle.

In recent decades, studies focused on disturbed circadian biological rhythms (chronopathology), which in terms of potential negative consequences, represent significant interest for researchers to disclose mechanisms for a variety of pathologies from cells to the whole organism. The role of the hormone melatonin to regulate sleep-wake cycle is linked to its therapeutic application in disorder characterized by desynchronized circadian rhythms, including jet-lag syndrome observed during the crossing of several time zones, work in night regime, long-term light exposure at night, etc. Experimental studies revealing that exogenous melatonin has chronotropic activity support the hypothesis that rather regulated by light secretion of this hormone is the main synchronizing signal that coordinates internal physiological functions with light-dark cycle. Exposure to light at night suppresses melatonin release causing a decrease in its synchronizing activity on cells in the periphery. Two experimental approaches for melatonin deficit are described in the literature, chronic constant light (CCL) exposure and pinealectomy, both are characterized by disturbed circadian rhythms of the endocrine and metabolic functions in the body according to the light-dark cycle [2-4]. Both approaches are also considered to model melatonin-deficient hypertension associated with myocardial, vascular and renal dysfunction [2]. Recently, we reported that CCL exposure in rats resulted in changes in behavioural responses associated with impaired circadian rhythm of melatonin and corticosterone secretion. In the present study, we aimed to compare two models of melatonin deficiency and specifically the role of abolished diurnal variations of melatonin and corticosterone for disturbed emotional status in rats.

Materials and methods. Subjects. Thirty two-day-old male Wistar rats (breeding house of the INB, BAS) were maintained in standardized laboratory conditions: temperature 21 °C, humidity 50–60%, artificial light/dark 12:12 regime (light on at 06:00 a.m.) with an exception of CCL group. A period of 3-weeks of exposure to constant light (100–120 lux each cage) was applied to a CCL-group according to our previous protocol [3]. Surgical removal of the pineal gland was executed following the protocol described by HOFFMAN and REITER [5]. The ex-

periments were performed in accordance with the European Communities Council Directives of 24 November 1986 (86/609/EEC).

Elevated-plus maze test. The apparatus consisted of two open arms (50×10 cm), two enclosed arms ($50 \times 10 \times 50$ cm), and a central platform (10×10 cm) elevated 50 cm above the floor level. At the beginning of the test, the rat was placed on the central platform facing an open arm. The test lasted 5 min. The calculated standard measures were: (1) total distance traveled (cm); (2) time (s) spent and (3) numbers of entries in the open arms.

Saccharin preference test. The test for evaluating of anhedonia was performed as described earlier [3]. The test consisted of two 12-h time intervals where one bottle filled with water (100 ml) and another with a saccharine solution (100 ml) were weighed after 12 h or replaced by a second pair of pre-weighed bottles. Taste preference was expressed as a percentage of consumed volume of saccharine solution during a 12-h period.

Forced swim test (FST). Depressive-like behaviour of rats measured as immobility time (s) was performed in an adapted version of the test, as was described earlier in a plastic container (height 60 cm, diameter 45 cm) filled to 30 cm with 21–24 °C water within a 5-min period [4]. The increased immobility indicated despair-like behaviour.

Statistical analysis. The results were presented as mean $\pm$ SEM. Experimental data were evaluated by Student's *t*-test. $P < 0.05$ was accepted with statistically significant differences.

Results and discussion. The effect of melatonin deficiency on motor activity and anxiety behaviour is demonstrated in Fig. 1A,B,C. The exposure to CCL did not influence motor activity ($p > 0.05$) but was accompanied by increased anxiety, measured with the time ($p = 0.0112$) and the number of entries ($p = 0.0029$) in the aversive open arms of the EPM. These results agree with our previous report and other literature data with this model [3,6]. Contrariwise, the pinealectomy significantly elevated the total distance traveled ($p = 0.0110$), the time spent ($p = 0.0383$) and the number of entries ($p = 0.0099$) in the open arms. These “anxiety-resistant” responses might reflect disinhibitory and impulsive behaviour rather than low anxiety [7]. Indeed, recently we reported that CCL model is characterized by increased latency to feed in a new environment in the novelty suppressed feeding test, which indicates a level of anxiety [4]. Although KARAKAŞ et al. [8] suggested that the pineal gland is not crucial for anxiety behaviour, they showed that melatonin deficit could cause an increased entrance frequency to the centre of the OF and time spent in the open arms of the EPM in rats.

Chronic constant light caused a phase-dependent anhedonic behaviour with a significantly diminished saccharine preference during the dark period ($p = 0.03$) (Fig. 2A). In addition to the higher preference for a sweet solution during the light phase, LL rats showed diurnal fluctuations ($p = 0.0351$) of liquids intake with higher consumption during the inactive and subjective light phase. How-

Fig. 1. Effect of exposure to chronic constant light (CCL) and pinealectomy on elevated plus-maze test: A) total distance travelled; B) time in open arms; C) entries in open arms. Data are presented as means $\pm$ S.E.M. Groups: LD – rats on 12:12 light/dark cycle; LL – rats on CCL regime; Sham – control group with sham surgery; Pin – rats with removed pineal gland. ** $p = 0.0112$, $p = 0.0029$, $p = 0.0110$, * $p = 0.0383$, ** $p = 0.0099$, respectively

ever, depressive-like behaviour was expressed without diurnal variations in rats with pinealectomy with significantly decreased affinity to saccharine consumption during the light ($p = 0.0331$) and the dark ($p = 0.048$) phase, respectively.

The two models of melatonin deficiency were characterized by a despair-like behaviour of rats measured as increased immobility in the FST ($p = 0.0378$ LL group vs LD group; $p = 0.0288$ Pin group vs Sham group) (Fig 2B). This depressive-like behaviour developed by Pin group is consistent with the findings of ZAHRA et al. [9] that emotional disturbance assessed in the FST is sex-dependent in rats with pinealectomy.

Fig. 2. Effect of exposure to chronic constant light (CCL) and pinealectomy on A) preference to saccharine solution (%) in the saccharine preference test, and B) immobility time (s) in the forced swimming test. Data are presented as means $\pm$ S.E.M. * $p = 0.03$; $p = 0.0351$; $p = 0.0331$; $p = 0.048$; $p = 0.0378$; $p = 0.0288$, respectively. Abbreviations as in Fig. 1

Figure 3A shows the effect of CCL and pinealectomy procedure on the circadian rhythm of plasma melatonin levels. In line with our recent report [3], plasma melatonin followed diurnal fluctuations with increased levels during the inactive and subjective dark period (22:00 and 04:00 h) in both control groups (LD and Sham). The two procedures, CCL and removal of the pineal gland, caused a melatonin deficiency related to a flattened pattern without rhythmic hormonal fluctuations and a lack of diurnal rhythm of hormonal levels in plasma ($p < 0.05$) (22:00 and 04:00 h vs LD and Sham group, respectively). These findings agree with our recent study [4].

The two models of melatonin deficit were characterized by high plasma corticosterone levels during the light period compared to control groups ($p < 0.001$ LL group vs LD group; $p = 0.017$ Pin group vs Sham group) with disrupted diurnal fluctuations suggesting an impaired regulatory control of hypothalamic-pituitary-adrenal cortex (HPA) axis. It is accepted that the disturbance in the circadian rhythm of the release of important hormones, including melatonin and cortisol, is implicated in the symptomatic nature of affective disorders, such as depres-

Fig. 3. Effect of exposure to chronic constant light (CCL) and pinealectomy on A) circadian rhythm of plasma melatonin level (ng.ml⁻¹), and B) diurnal variations of plasma corticosterone levels. Each group consisted of at least $n = 8$ rats decapitated every 6 h (A) or at 10:00 and 22:00 h, respectively (B). Results are expressed as means $\pm$ SEM. * $p < 0.05$ (A), $p < 0.001$; $p = 0.017$ (B). Abbreviations as in Fig. 1

sion and anxiety [10,11]. Patients with depression are characterized by disrupted rhythms of both melatonin and cortisol [12–14].

Conclusion. Our findings revealed that the two models of melatonin deficiency, CCL and pinealectomy might recapitulate several neurobiological alterations associated with melancholic depression. However, they are characterized by a model-specific difference in emotional status, possibly not associated with an impaired HPA axis regulation.

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