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ORIGINAL ARTICLE

Effects of melatonin on 24-h rhythms of neuroendocrine and immune changes in Freund's adjuvant-induced arthritis

Ana I. Esquifino[1] & Daniel P. Cardinali[2]

1. Departamento de Bioquimica y Biologia Molecular III, Facultad de Medicina, Universidad Complutense, Madrid, Spain.
2. Departamento de Fisiologia, Facultad de Medicina, Universidad de Buenos Aires, Argentina.

Correspondence to: Dr. Ana I. Esquifino, Departamento de Bioquimica y Biologia Molecular III, Facultad de Medicina, Universidad Complutense, Ciudad Universitaria, 28040 Madrid, Spain
TEL: +34 91 394 1678; FAX: +34 91 394 1678
E-mail: pelayos@eucmax.sim.ucm.es

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Abstract

Old rats had significantly lower values of the amplitude of pineal melatonin content at any time point studied. Complete Freund's adjuvant administration reduced the amplitude of melatonin rhythm. On day 18 of arthritis development, decreased pineal melatonin contents were also seen in young rats. On day 18, a global depressive effect of arthritis development on pineal melatonin content was found. However, prolactin levels increased in old as compared to young rats. Freund's adjuvant administration brought about changes in the secretory pattern of prolactin in young and old animals that are opposite to those described for melatonin. Cell proliferation as assessed by ornithine decarboxylase activity exhibited significant 24-h variations with maximal activity of the enzyme during daily hours, acrophases varying from 13:15 to 16:52 h (lymph nodes). The mesor and amplitude of ornithine decarboxylase activity were lowest in old rats. Melatonin (100 μg) augmented amplitude of daily rhythm in submaxillary lymph node ornithine decarboxylase activity significantly. These data indicate that melatonin and prolactin change differentially during arthritis development in young and old animals, perhaps indicating opposite effects in the induction and maintenance of adjuvant-arthritis. The changes are compatible with the values of ornithine descarboxylase activity.

Introduction

Melatonin is a versatile hormone that exerts a variety of functions. Among them, immunomodulation has emerged as a major effect in vertebrates. Melatonin rhythm appears to be an important efferent pathway of the central nervous system (CNS) to synchronize the immune system. This pathway allows the immune cells to define time intervals on 24-h and stational and annual cycle bases. This may be an important event in the possible role of melatonin in the appearance and development of autoimmune diseases.

Effect of complete Freund's adjuvant on melatonin and prolactin rhythms during adjuvant-arthritis development

Groups of young and old rats were studied on day 18 after mycobacterium adjuvant injection. Pineal melatonin content exhibited significant 24-h variations in all groups studied, with its maximal activity at night, acrophases varying from 00:06 to 01:04 h. Old rats had the lowest pineal melatonin levels. On day 18, a global depressive effect of arthritis development on pineal melatonin content was found (Table 1).

These results indicate an age-dependent, significant depression of pineal melatonin synthesis in rats, concomitant with a decrease in amplitude of immune cell proliferation and autonomic neural activity. These data are compatible with the previously reported neuronal degeneration at the suprachiasmatic [1], together with a decrease in number of pinealocytes [2] and in the amplitude of melatonin rhythm [3–7] occurring during aging.

As far as the prolactin rhythm, it was of higher amplitude than that observed in young animals, in agreement with previous reports showing that prolactin increases during aging [8]. Freund's adjuvant administration brought about changes in the secretory pattern of prolactin in young and old animals (Table 2). The maxima in plasma prolactin levels of young animals was shift advanced to 17:00 h and the peak was maintained at 21:00 h as well. In old animals a similar effect was observed although a marked increase in plasma prolactin levels was observed at 08:00 h.

The changes observed on day 18 after Freund's adjuvant administration are different from those observed during the early phase of arthritis development in experiments performed during the winter [7] or in the spring [8, this study].

Table 1. Twenty-four-hour changes in pineal melatonin content, in young and old male rats 18 days after Freund's adjuvant or adjuvant vehicle injection

	YOUNG		OLD	
Time (h)	Veh ACF	ACF	Veh ACF	ACF
09:00	0.30 ± 0.05	0.20 ± 0.05	0.47 ± 0.30	0.23 ± 0.04
13.00	0.60 ± 0.20	0.25 ± 0.03	0.35 ± 0.03	0.44 ± 0.20
17:00	0.35 ± 0.05	0.20 ± 0.06	0.22 ± 0.05	0.40 ± 0.03
21:00	2.80 ± 0.60	2.09 ± 0.06	2.50 ± 1.00	1.62 ± 0.40
01:00	3.70 ± 0.50	4.80 ± 0.08	4.31 ± 1.50	2.00 ± 0.50
05:00	2.10 ± 0.70	1.44 ± 0.60	1.70 ± 0.50	0.73 ± 0.04
09:00	0.20 ± 0.07	0.15 ± 0.06	0.28 ± 0.09	0.28 ± 0.06

Table 2. Twenty-four-hour changes in serum prolactin values, in young and old male rats 18 days after Freund's adjuvant or adjuvant vehicle injection.

	YOUNG		OLD	
Time (h)	Veh ACF	ACF	Veh ACF	ACF
09:00	2042 ± 152	1591 ± 144	3685 ± 250	5000 ± 589
13.00	1089 ± 136	946 ± 113	2374 ± 83	2258 ± 265
17:00	1355 ± 110	4039 ± 132	1680 ± 42	4613 ± 790
21:00	4146 ± 141	3976 ± 152	6638 ± 150	5440 ± 810
01:00	1251 ± 145	1578 ± 130	3783 ± 100	3260 ± 401
05:00	1216 ± 66	1762 ± 153	3313 ± 140	2845 ± 360
09:00	2042 ± 53	1591 ± 144	3285 ± 250	5472 ± 1200

The data indicated that as arthritis progressed, the disease differentially affected the circadian pattern of prolactin, thus suggesting a role of the hormone in the induction and progress of autoimmune diseases [9]. It is of interest to note that melatonin and prolactin change differentially during arthritis development in young and old animals, perhaps indicating opposite effects in the induction and maintenance of adjuvant-arthritis.

Effect of exogenous melatonin administration on the development of adjuvant-arthritis in young and old rats

We have demonstrated that complete Freund's adjuvant administration produces an increase in the paw volume and also that melatonin induced the inflammatory response in the hind paws in young animals [10]. In old rats melatonin restored the inflammatory response in the hind paws of Freund's adjuvant-injected rats to the levels found in young animals [11].

During the immune reaction, cell proliferation as assessed by ornithine decarboxylase activity, augmented significantly. This enzyme exhibited significant 24 hour variations with maximal activity during daily hours, acrophases varying from 13:15 to 16:52 h (lymph nodes).

The mesor and amplitude of ornithine decarboxylase activity were lowest in old rats.

Melatonin (100 μg) augmented amplitude of daily rhythm in submaxillary lymph node ornithine decarboxylase activity significantly (Tables 3 and 4). In the case of splenic ODC activity the results were similar to those observed in the submaxillary lymph nodes. However, in this case, the lower dose of melatonin (10 μg) was also effective in restoring the amplitude of ODC rhythm in old rats [10, 11].

The changes in the cell proliferation may reflect an activation of T-lymphocytes as adjuvant-induced arthritis is a specific autoimmune disease that seems to be dependent on these cells, and on the strain of rat used [3, 12].

In a previous study we reported that at the early phase after, Freund's adjuvant injection (3 days after) the increased serum ACTH and prolactin levels found were accompanied by minor modifications of 24-h rhythmicity of hormone secretion [7]. We also found that Freund's adjuvant-injected rats exhibited abolition of 24-hour variation in circulating TSH. Presumably, the chronic stress condition given by mycobacterial adjuvant injection is instrumental in changing a number of circadian rhythms at this early phase of disease.

The results of this study indicate the existence of an age-dependent, significant effect of immune-mediated inflammatory response on 24-h rhythms in cell proliferation. During the immune reaction, lymph node and splenic ornithine decarboxylase, an index of immune cell proliferation, augmented 8–10-fold, with progressively smaller amplitude of daily variations as arthritis developed. In every case, mesor and amplitude of 24-h rhythm in ornithine decarboxylase activity were lowest in old rats. All these effects were reversed or significantly counteracted by melatonin administration.

In other studies we reported that adrenergic and cholinergic markers follow 24-h fluctuations and that the maxima occurred at night (adrenergic) or during the day (cholinergic) [13]. As it happened for ODC, both markers showed the lowest amplitude in their rhythms and melatonin treatment restored the amplitude of rhythms in old animals treated with the adjuvant.

Taking into consideration all these parameters studied, it is of interest to note that melatonin was remarkably effective in counteracting the significant decrease of immune cell responsiveness in lymph nodes and spleen found in aged animals and that this responsiveness was related to changes in the peripheral innervation of the submaxillary lymph nodes and spleen.

TABLE 3. Twenty-four-hour changes in submaxillary lymph node ornithine decarboxylase activity, in young male rats 18 days after Freund's adjuvant (ACF) or adjuvant vehicle (Veh ACF) injection, and receiving daily injections of 100 µg of melatonin (MEL) or vehicle (Veh MEL) from day 1 to day 17.

	Veh ACF		ACF	
Time (h)	Veh MEL	MEL	Veh MEL	MEL
09:00	30 ± 0.9	27.5 ± 5.0	180 ± 30	290 ± 20
13.00	60 ± 5.0	75.0 ± 10	270 ± 20	540 ± 60
17:00	43 ± 7.5	65.0 ± 7.0	280 ± 30	510 ± 65
21:00	14 ± 1.5	27.5 ± 3.5	200 ± 10	300 ± 50
01:00	29 ± 1.0	22.5 ± 3.5	150 ± 10	290 ± 40
05:00	22 ± 3.0	30.0 ± 5.0	140 ± 40	280 ± 20
09:00	35 ± 0.5	29.5 ± 3.5	160 ± 20	295 ± 25

TABLE 4. Twenty-four-hour changes in submaxillary lymph node ornithine decarboxylase activity, in old male rats 18 days after Freund's adjuvant (ACF) or adjuvant vehicle (Veh ACF) injection, and receiving daily injections of 100 µg of melatonin (MEL) or vehicle (Veh MEL) from day 1 to day 17.

	Veh ACF		ACF	
Time (h)	Veh MEL	MEL	Veh MEL	MEL
09:00	33.0 ± 6.0	34.0 ± 5.0	150 ± 30	280 ± 20
13.00	31.5 ± 3.5	67.0 ± 8.0	210 ± 50	530 ± 70
17:00	35.5 ± 5.0	65.0 ± 5.0	205 ± 15	508 ± 62
21:00	12.5 ± 5.0	33.5 ± 3.0	200 ± 15	303 ± 50
01:00	15.0 ± 2.5	24.1 ± 3.0	180 ± 30	290 ± 25
05:00	17.5 ± 2.0	23.2 ± 4.5	160 ± 20	270 ± 35

Previous observations indicate that any experimental procedure that inhibits melatonin synthesis and secretion induces immunodepression that is counteracted by exogenous melatonin administration [14–17]. In vivo, melatonin displays an immunoenhancing effect, particularly apparent in immunodepressive states. Melatonin administration augmented several immune parameters. Therefore, our data are compatible with the view that melatonin exerts a potent immunoenhancing activity in rats.

Melatonin effect may not always be beneficial. Hansson et al. [18] examined whether the enhancing effect of constant darkness on autoimmunity to type II collagen was due to melatonin. Melatonin administration (1 mg/kg) performed daily in the afternoon for 10 days after collagen injection induced a more severe arthritis. Accordingly, pinealectomy in two strains of mice immunized with rat type II collagen and kept in constant darkness reduced the severity of the arthritis as shown by a slower onset of the

disease, a less severe course of the disease (reduced clinical scores) and reduced serum levels of anti-collagen II antibodies [19].

Our present observations kept in pace with those results in which an inflammation-promoting effect of 100 μg melatonin could be demonstrated in young rats injected with mycobacterial adjuvant. In contrast, melatonin administration (10 and 100 μg) to old rats restored the inflammatory response of hind paws of Freund's adjuvant-injected rats to levels found in young rats. Therefore, high levels of melatonin in young animals may stimulate the immune system and cause exacerbation of both autoimmune collagen II and mycobacterial arthritis. However, the nature of the mechanisms involved in the immunomodulatory activity of melatonin remains highly speculative. A direct effect of the hormone on the immune cells is suggested as specific membrane binding sites for melatonin on these cells were found [20]. Indirect effects of the hormone cannot be excluded [21].

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