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Effects of pineal body and melatonin on lymphocyte proliferation and dinoprostone production in rat spleen

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AIM: To determine the effects of pineal body and melatonin (Mel) on lymphocyte proliferation and dinoprostone production in rat spleen. **METHODS:** Pinealectomy (PE), lymphocyte proliferation assay and dinoprostone radioimmunoassay were used. **RESULTS:** A circadian rhythm of splenic lymphocyte proliferation which peaked at 22:00 was obliterated by PE in rats. PE led to an impairment of lymphocyte proliferation and an increase of dinoprostone production, which were restored by ip Mel 10 $\mu g \cdot kg^{-1} \cdot d^{-1}$ at 16:00 for 7 d. Mel promoted lymphocyte proliferation and inhibited dinoprostone production in intact rats. A negative correlation between the change in lymphocyte proliferation and dinoprostone production was seen. **CONCLUSION:** The pineal body and its main hormone Mel play a regulatory role in circadian lymphocyte proliferation which is related to dinoprostone production in rat spleen.

KEY WORDS pineal body; melatonin; spleen; lymphocyte; dinoprostone

The pineal body has been recognized as an important component of the neuroendocrine system regulating circadian rhythmicity. The release of norepinephrine from postganglionic sympathetic fibers originating in the superior cervical ganglion stimulates the production of the pineal hormone melatonin (Mel) via its binding to adrenoceptors located on pinealocytes. Mel conveys the influence of the light-dark cycle on body physiology, thus affecting the regulation of the neuroendocrine and immune systems[1,2]. As shown by several studies, abrogation of the cyclicity of the pineal secretion by pinealectomy (PE) or by permanent lighting leads to impairment of cellular and humoral immune responses, whereas exogenous Mel enhances antibody production, helper inducer T lymphocyte activity and IL-2 production[3-5]. We have demonstrated that the pineal body and Mel play an important role in inflammatory and immune respones[6,7]. But their effects on circadian rhythm of lymphocyte proliferation have not been found. In this article, we studied the effects of the pineal body and Mel on lymphocyte proliferation and dinoprostone production in the spleen of adult male rats to determine whether there is a circadian rhythm of lymphocyte proliferation controlled by pineal body and this immunoregulatory effect is mediated by dinoprostone changes in the spleen.

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MATERIALS AND METHODS

Animals Sprague-Dawley rats (♂, $n = 110$, 3-4 month old, 251 ± s 50 g) were provided by the Animal Center of Anhui Medical University. Rats were maintained under laboratory conditions with 12-h light (6:00-18:00) and 12-h darkness at 22 ± 1 C with free access to food and tap water.

Reagents and chemicals Mel, purchased from Sigma Chemical Co (St Louis MO), was dissolved in ethanol and then diluted with normal saline to the final concentration of 2 % ethanol. Concanavalin A (Con A) and lipopolysaccharides (LPS), from Sigma, were prepared at a concentration of 1 $g \cdot L^{-1}$ and stored at -20 C. Medium RPMI-1640 (Gibco Laboratories) was supplemented with HEPES buffer 10 $mmol \cdot L^{-1}$,

penicillin 10^5 IU·L^{-1}, streptomycin 100 mg·L^{-1}, glutamine 2 mmol·L^{-1}, 2-mercaptoethanol 50 μmmol·L^{-1}, and 10 % new-born bovine serum and was adjusted to pH 7.4. [^{3}H]Thymidine (TdR) 37 GBq·L^{-1} was purchased from Chinese Academy of Atomic Energy Science, Beijing. Dinoprostone radioimmunoassay kit was obtained from Chinese Academy of Medical Sciences, Beijing.

Pinealectomy Pinealectomy was done under 10% chloral hydrate anesthesia. The area of the dorsal surface of the brain around the confluence of the transverse and sagittal sinuses was exposed and the dura mater ruptured at a point just lateral and anterior to sinus confluence. Fine forceps were then inserted beneath the confluence at an angle of 45° to the horizontal and withdrawn enclosing the pineal, thus rupturing the pineal stalk[8]. Sham-pinealectomy consisted of a similar procedure, but the forceps were kept closed during the insertion so that no tissue was removed.

Lymphocyte proliferation assay The spleens were excised from rats after decapitation. Single cell suspensions were prepared by grinding gently against steel mesh in cold RPMI-1640 under aseptic condition. Erythrocytes were lysed with 0.83 % NH_4Cl buffered solution, washed twice in Hank's solution, and centrifuged (500 × g) for 10 min. The cells were then resuspended at 1×10^{10} cells·L^{-1} in RPMI-1640 media. Cell viability exceeded 95 % as determined by trypan blue exclusion technic. Splenocytes 1×10^6 per well in RPMI-1640 media were seeded into 96-well flat-bottom microtiter plates in the presence of Con A (5 mg·L^{-1}) or LPS (5 mg·L^{-1}). The cultures were incubated for 48 h in a humidified 5 % CO_2 environment at 37℃. [^{3}H]TdR 7.4 kBq per well was added 6 h before the termination of culturing. The cells were harvested on type-69 glass fiber filters. The radioactivities of the filters were counted in a FJ-2107 liquid scintillation counter [9].

Dinoprostone radioimmunoassay Spleen (30 mg) was homogenized in 1 ml of saline acidified to pH 3—4. EtAc 2 ml were added to the homogenate and vortexed for 30 s. The homogenate was then centrifuged at 1000 × g for 20 min. Another 2 ml of EtAc were added and centrifuged. The EtAc layer of the homogenate was evaporated under N_2[9]. The radioimmunoassay was performed according to instruction provided in the kit. Concentration of dinoprostone was expressed as μg/ kg of tissue.

Statistics Correlation coefficient were calculated using Casio fx-4200p calculator. Statistical analyses were carried out using *t* tests.

RESULTS

Effect of PE on circadian rhythm of splenic lymphocyte proliferation Rats were killed at 4-h intervals around the clock beginning at 6:00 on d 8 after PE. In intact and sham-pinealectomized rats there existed a clear circadian rhythm in splenic T- and B-lymphocyte proliferations which peaked at 22:00. PE almost obliterated the circadian rhythm, and the proliferation were lower than that in the intact rats ($P<0.01$) (Fig 1).

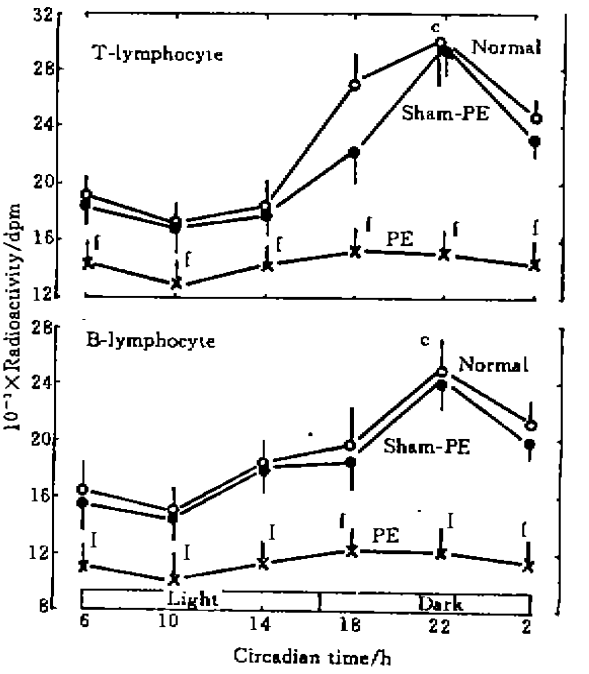

Fig 1. Con A- and LPS-induced splenic T- and B-lymphocyte proliferation in normal rats, sham-pinealectomized (PE) rats and PE rats on d 8 after PE. $n=5$, $\bar{x}\pm s$. $^cP<0.01$ *vs* other time. $^fP<0.01$ *vs* the same time in normal and sham-PE rats.

Effects of PE and Mel on lymphocyte proliferation and dinoprostone production

Mel 10 $\mu g \cdot kg^{-1}$ was injected ip daily at 16:00 for 7 consecutive days from d 8 after PE. It counteracted the decreased T- and B- lymphocyte proliferations induced by PE. PE enhanced splenic dinoprostone production, which was antagonized by Mel treatment. Mel promoted lymphocyte proliferation and inhibited dinoprostone production in intact rats (Tab 1). There was an inverse relationship between T-lymphocyte proliferation and dinoprostone production ($\hat{Y} = 25249 - 332X$, $r = -0.81$, $P<0.01$) or B-lymphocyte proliferation and dinoprostone production ($\hat{Y} = 22477 - 283X$, $r = -0.79$, $P<0.01$).

Tab 1. Effects of pinealectomy (PE) and ip melatonin (Mel) at 16:00 on Con A (5 mg·L^{-1})- or LPS (5 mg·L^{-1})- induced lymphocyte proliferation and dinoprostone production in rat spleen. Lymphocyte proliferation was measured by radioactivity of [^{3}H]TdR uptaken by splenocyte and dinoprostone was detected by radioimmunoassay. $n = 5$, $\bar{x} \pm s$. $^cP<0.01$ *vs* control, $^fP<0.01$ *vs* PE.

	Dose/ $\mu g \cdot kg^{-1} \cdot d^{-1} \times 7$	Radioactivity/dpm Con A	LPS	Dinoprostone/ $\mu g \cdot kg^{-1}$
Control	NS	17325±1716	15732±2335	14.3±2.2
Sham-PE	NS	15592±2595	14022±1475	15.4±2.4
PE	NS	8865±1022^c	8925±1809^c	52.0±10.1^c
PE+Mel	10	13011±866^f	16603±1975^f	21.5±3.7^f
Mel	10	29428±2553^c	26003±2300^c	6.5±1.5^c

DISCUSSION

Our present study provides envidence of a circadian rhythm pattern peaking at 22:00 in the proliferation of splenic lymphocytes obtained from intact rats. This rhythm almost parallels the Mel rhythm pattern[2]. Our results show that abrogation of the Mel rhythm pattern by PE led to an obliteration of the circadian rhythm and an impairment of splenic lymphocyte proliferation in rats. Mel treatment could restore the proliferation in PE rats and promote it in intact rats. These observations strongly suggest the pineal body plays an important regulatory role in circadian lymphocyte proliferation via the circadian secretion of Mel.

The chemical structure of Mel closely resembles that of indometacin. Mel 0.01 — 10 $\mu mol \cdot L^{-1}$ added into medial basal hypothalamus media dose-dependently inhibited dinoprostone production from [^{14}C] arachidonic acid[9]. So Mel may be one of the cyclooxygenase inhibitors. Our results show that dinoprostone production in the spleen was increased by PE, which could be reversed by Mel treatment. Mel also inhibited dinoprostone production in the spleen of intact rats. Negative correlation between the change in dinoprostone production and lymphocyte proliferation was seen. These indicate effect of the pineal body and Mel on splenic lymphocyte proliferation is related to dinoprostone production.

Taken together, the results from the present study demonstrate that pineal body and Mel play an important regulatory role on circadian lymphocyte proliferation. Althrough it was proposed that immunoregulatory effects of the pineal body and Mel were mediated by opioid[3] and adrenal[10] system, and a direct effect of Mel on lymphocytes has been found[11,12], our present study show that this effect of the pineal body and Mel is related to dinoprostone production in rat spleen.

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松果体和褪黑激素对大鼠脾淋巴细胞增殖和地诺前列酮产生的影响

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目的：研究松果体和褪黑激素对大鼠脾淋巴细胞增殖及地诺前列酮产生的影响。 **方法**：采用松果体切除术，淋巴细胞增殖反应测定及地诺前列酮放射免疫检定法。 **结果**：大鼠脾淋巴细胞增殖反应存在昼夜节律。松果体切除后，此昼夜节律消失，脾淋巴细胞增殖反应降低，而脾脏地诺前列酮增加。16:00 ip 褪黑激素 (Mel) 10 $\mu g \cdot kg^{-1} \cdot d^{-1}$ 连续7 d 能恢复之，且促进正常大鼠脾淋巴细胞增殖反应，抑制脾地诺前列酮的产生。 **结论**：松果体 Mel 促进大鼠脾淋巴细胞增殖反应与抑制脾脏地诺前列酮的产生有关系。

关键词 松果体；褪黑激素；脾；淋巴细胞；地诺前列酮