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Effect of pineal body and melatonin on chemiluminescence of peritoneal macrophages via hypothalamus in rats

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AIM: To study whether the hypothalamus was a major relay for the action of melatonin (Mel) on peritoneal macrophage (PM∅) function. **METHODS**: Pinealectomy, luminol-dependent chemiluminescence (CL), hypothalamic dinoprostone radioimmunoassay, and intrahypothalamic injection of Mel were done in rats kept under light : dark 12 h : 12 h. **RESULTS**: CL value of PM∅ was decreased and hypothalamic dinoprostone content was elevated after pinealectomy, which were restored by Mel (10 $\mu g\ kg^{-1}\ d^{-1}$ ip at 16 : 00 for 7 d). The same treatment of Mel increased CL value of PM∅ and depressed hypothalamic dinoprostone production in intact rats. CL value of PM∅ showed negative relation to hypothalamic dinoprostone with $r = -0.78$ ($P < 0.01$). Intrahypothalamic injection of Mel (2 μg) enhanced CL value of PM∅ in normal and pinealectomized rats. **CONCLUSION**: The hypothalamus is a main site of pineal Mel action upon PM∅ function.

KEY WORDS pineal body; melatonin; peritoneal macrophages; chemiluminescence; hypothalamus; dinoprostone

The pineal body, as a neuroendocrine transducer by translating the most basic environmental information into signals that modulates neuroendocrine mechanisms[1,2], has been postulated to be involved in the functional connection between the immune and central nervous systems[3]. The immunoregulatory effects of melatonin (Mel), the major pineal hormone, were widely demonstrated by immuno-enhancing and oncostatic activity in animals. They play a modulatory role on inflammatory and immune responses[4-6]. However, the sites of Mel action are less known. The brain is the primary locus where Mel acts, and high affinity binding sites for Mel are found in several brain regions, particularly the hypothalamus[7,8]. Therefore, the present study was to investigate whether the hypothalamus was a major site for Mel action on macrophage functions.

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MATERIALS AND METHODS

Rats Forty-five Sprague-Dawley rats (♂, 3—4 months old, 234 ± *s* 46 g) were provided by Animal Center of Anhui Medical University. Rats were kept under light from 6 : 00 to 18 : 00 daily at 22±2 ℃ with free access and tap water.

Reagents and chemicals Mel, purchased from Sigma Chemical Co, was dissolved in 100 % ethanol and then diluted with normal saline to a final concentration of 2 % ethanol. Luminol (Sigma) was dissolved in triethylamine and phosphate-buffered saline (PBS) to a concentration of 2 g L^{-1}. Zymosan (Sigma) was suspended in PBS, boiled for 15 min, and then resuspended in DMEM (Gibco) at 50 g L^{-1}. Opsonized zymosan was made by incubating boiled zymosan with equal volume of fresh autologous serum at 37 ℃ for 30 min and then resuspending in PBS. Dinoprostone radioimmunoassay kit was obtained from the Chinese Academy of Medical Sciences, Beijing.

Pinealectomy (PE) The operation was doner[9]. After 7 d, rats were injected ip with Mel 10 $\mu g\ kg^{-1}\ d^{-1}$ or vehicle for 7 d.

Chemiluminescence (CL) Rat peritoneal macrophage (PM∅) suspensions were prepared at a

concentration of 2×10^9 L^{-1}. After 500 μL of PMØ suspensions were incubated at 37 ℃ for 20 min, they were placed in the measuring chamber of a SHG-1 bio- or CL analyzer (Shanghai), 100 μL of freshly prepared luminol 0.1 mmol L^{-1} and 100 μL of zymosan particles 5 g L^{-1} were added. CL was continuously recorded by a computer and a printer connected to the CL machine[10].

Dinoprostone radioimmunoassay In 30 mg of hypothalamus[6].

Intrahypothalamic injection of Mel Rats were anesthetized with 10 % chloral hydrate (300 mg kg^{-1} ip) and stereotaxically implated (P 1.0, L 0.1, H 8.0)[11] with a chronic cannula guide tube fabricated from 22-gauge thin-wall stainless steel hypodermic needle and contained an indwelling stylus made from 27-gauge stainless steel wire. The guide tube was anchored on the skull. Eight days later, rats received intrahypothalamic injection of Mel at 16:00 for 7 d. For injection, a 10-μL Hamilton syringe was inserted into the guide tube so that its tip extended 0.5 mm into the brain tissue. Before injection, the syringe was washed in 70 % ethanol. Mel 2 μg was injected in 1 min and the syringe was remained for another 1 min. 1 μL of 1 % ethanol in saline (vehicle) was given for control injection.

A total of 20 implant operations were performed, and all rats recovered well. The hypothalamic area was frozen in liquid nitrogen, sectioned with a cryostat, fixed in cold acetone, and stained with crysyl violet and eosin. By reference to an atlas[11], in all but 2 rats the injection cannulae were present in the vicinity of the suprachiasmatic and anterior hypothalamic area. The 2 rats showing injection sites in the thalamus were discarded.

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

RESULTS

Mel 10 μg kg^{-1} counteracted the decreased PMØ CL and increased hypothalamic dinoprostone production induced by PE. The same treatment of Mel increased PMØ CL and the inhibited dinoprostone production in intact rats (Tab 1). There was an inverse relationship between PMØ CL and hypothalamic dinoprostone production ($\hat{Y} = 5580 - 80X$, $r = -0.78$, $P < 0.01$).

Tab 1. Effects of pinealectomy (PE) and ip melatonin (Mel) at 16:00 on dinoprostone production in hypothalamus and chemiluminescence (CL) of peritoneal macrophages in rats. $n = 5$, $\bar{x} \pm s$. **[c] $P < 0.01$ vs control, [f] $P < 0.01$ vs PE.**

	Dose/ μg kg^{-1} d^{-1} × 7 d	CL/ cpm	Dinoprostone/ ng g^{-1}
Control	—	3 039±506	20±4
Mel	10	6 413±1075[c]	11.2±2.1[c]
Sham-PE	—	2 988±330	19.3±2.4
PE	—	1 166±215[c]	58±11[c]
PE+Mel	10	3 428±204[f]	23.4±2.2[f]

Intrahypothalamic injection of Mel (2 μg) increased PMØ CL in intact rats compared to those given vehicle injections. It also restored the decreased PMØ CL in pinealectomized rats (Tab 2).

Tab 2. Effects of intrahypothalamic injection of Mel 2 μg for 7 d on CL of peritoneal macrophages in normal and pinealectomized (PE) rats. $\bar{x} \pm s$. **[c] $P < 0.01$ vs control, [f] $P < 0.01$ vs PE.**

	n	Dose/ μg d^{-1} × 7 d	CL/ 10^{-3} × cpm
Vehicle	4	—	1.86±0.24
Mel	5	2	3.0±0.6[c]
PE	5	—	0.72±0.21[c]
PE+Mel	4	2	1.57±0.27[f]

DISCUSSION

Our present study demonstrated that dinoprostone content in the hypothalamus was increased by PE, which could be reversed by ip Mel. Mel also decreased dinoprostone production in the hypothalamus in intact rats. Hypothalamic prostaglandins play an important role in regulating pituitary function, *ie*, microinjection of prostaglandins in the

hypothalamus can effectively stimulate the release of LH, FSH, PRL, GH, and ACTH, etc[12]. So pineal Mel might modulate pituitary function via hypothalamic prostaglandins, thus affecting neuro-endocrino-immune network. Our result showed an inverse relationship between PMØ CL and hypothalamic dinoprostone production, suggesting that pineal Mel might regulate macrophage functions via hypothalamus. Intrahypothalamic injection of Mel enhanced PMØ CL in intact and pinealectomized rats. These observations indicated that the hypothalamus was the main site of pineal Mel action on macrophage functions.

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435-437 13

松果体和褪黑激素通过下丘脑影响大鼠腹腔巨噬细胞化学发光

R965.2

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目的：研究松果体和褪黑激素(Mel)是否通过下丘脑影响腹腔巨噬细胞功能. **方法**：松果体切除术；腹腔巨噬细胞化学发光测定；下丘脑地诺前列酮放射免疫测定；下丘脑注射Mel. **结果**：松果体切除后腹腔巨噬细胞化学发光值降低，下丘脑地诺前列酮含量升高，16:00 ip Mel (10 $\mu g\ kg^{-1}\ d^{-1} \times 7$ d)可使其恢复，并升高正常大鼠腹腔巨噬细胞化学发光值，降低其下丘脑地诺前列酮含量. 腹腔巨噬细胞化学发光值与下丘脑地诺前列酮含量的变化存在负相关(相关系数 $r = -0.78$, $P < 0.01$). 于下丘脑注射Mel 2 μg，能提高正常大鼠和松果体切除大鼠腹腔巨噬细胞化学发光值. **结论**：下丘脑是松果体Mel影响腹腔巨噬细胞功能的主要作用部位之一.

关键词 松果体；褪黑激素；腹腔巨噬细胞；化学发光；下丘脑；地诺前列酮