ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Nov; 22 (11): 997 - 1002
http://www.chinaphar.com


# Effects of microinjection of melatonin and its receptor antagonists into anterior hypothalamic area on blood pressure and heart rate in rats[1]

DING Chao-Nan, CAO Yin-Xiang, ZHOU Li, ZHU Da-Nian[2], SHEN Zhong-Yuan[3], FEI Min-Yi[3], YU Ping[3]
(*Department of Physiology, Medical Center of Fudan University, Shanghai 200032*; [3]*Shanghai University of Traditional Chinese Medicine, Shanghai 200032, China*)

**KEY WORDS** melatonin; anterior hypothalamic nucleus; blood pressure; heart rate

## ABSTRACT

**AIM**: To examine the effects of microinjection of melatonin and its receptor antagonists into the anterior hypothalamic area (AHA) on blood pressure (BP) and heart rate (HR) in normotensive and stress-induced hypertensive rats. **METHODS**: Melatonin and its receptor antagonists were microinjected into the AHA, then BP, mean arterial pressure (MAP), and HR were synchronously recorded. **RESULTS**: Microinjection of melatonin produced a fall in MAP. Prazosin, an antagonist of melatonin $ML_2$ receptor, could not antagonize the depressive response induced by melatonin. While luzindole, a competitive antagonist of melatonin $ML_1$ receptor, was able to almost completely prevented the depressive response induced by injection of melatonin. **CONCLUSION**: Melatonin acts as a hypotensive factor and the effects are mainly due to activation of $ML_1$ receptors in rat brain, and the AHA may be one of the important central areas where melatonin can exert modulatory effects on BP and HR.

## INTRODUCTION

Melatonin, one of the pineal hormones, has been found to play a role in the mechanism of cardiovascular regulation. The nocturnal increase of melatonin seems to show an inverse temporal relation with the decrease of cardiovascular activities[1]. It has been shown that surgical removal of the pineal gland elevated blood pressure in anaesthetized rats[2] and a modest hypertension (20 mmHg) has been described in unanaesthetized rats after electrocoagulation of pineal gland[3]. A decrease in serum melatonin concentration in essentially hypertensive patients[4] or in spontaneously hypertensive rats (SHR)[5] has been reported. The hypotensive effect of melatonin has been observed in patients with essential hypertension[6] or in rats with spontaneous hypertension[7]. In rats, melatonin is able to prevent cardiac arrhythmia induced by ischemia and/or reperfusion[8], decrease arterial pressure and heart rate[9,10], regulate blood flow to the brain[11], and modify peripheral artery responsiveness to norepinephrine[12]. There are many reports related to the peripheral mechanism of the antihypertensive effects of melatonin, but only rarely do papers describe the mechanism in the central nervous system (CNS) where melatonin receptors were found[13]. It has been proposed that melatonin may act as an endogenous hypotensive factor, possibly by stimulating central inhibitory adrenergic pathways[6,14]. It has been reported that intravenous administration of melatonin decreased serotonin release in the anterior hypothalamic area(AHA) and resulted in sympathetic inhibition which led to hypotension in rats[10]. The AHA has been postulated as a site of action for melatonin[15]. Therefore, in the present study, we tested the effect of melatonin and its receptor antagonists microinjected into the AHA on blood pressure (BP) and heart rate (HR).

## MATERIALS AND METHODS

**Animal preparations** Experiments were performed on male Sprague-Dawley (SD) rats (Grade Ⅱ, 10 - 12 weeks old, 300 g ± 20 g, Animal Department of Medical Center of Fudan University, № 02-22-2). Rats were divided into two groups, one group animals were

[1] Project supported by the National Natural Science Foundation of China, № 39970277.
[2] Correspondence to Prof ZHU Da-Nian. Phn 86-21-6404-1900, ext 2405. Fax 86-21-6403-7222. E-mail dnzhu@shmu.edu.cn
Received 2001-04-24 Accepted 2001-08-22

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Nov; 22 (11)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

put individually into the cages provided with the electric grid floor through which electric foot-shocks of 75 – 150 V and 50 – 100 ms duration were delivered from a steady voltage power at an interval of 2 – 30 s varied at random and controlled by a computer. When the electric foot-shocks were given, the animals were frightened by both somatic and mental stress. In order to enhance the mental stress and avoid too much somatic stimulation, a noise (88 – 98 db, 50 – 100 ms) produced by a buzzer was combined synchronously to form a conditioned reflex. Once the conditioned reflex had been established, the animals were stimulated only by the noise instead of by foot-shocks. However, the conditioned reflex to the noise would attenuate gradually. In order to strengthen the conditioned reflex, the foot-shocks should be given at irregular intervals dependent on the rat response to the conditioned stimulation. The animals received the stress twice daily (2 h × 2), which lasted 15 d. The pretreatment of foot-shocks and noises increased the BP of the animals by about 3 kPa. Another group of the rats, as a control group, received the sham stress, they were put into the same cage at the same time, but without any foot-shock and noise. The BP of this group animals remained normal.

Then the two groups of rats were anesthetized with a mixture of urethane and chloralose (700 mg/kg and 35 mg/kg, ip). The trachea was intubated first with a polyethylene tube, and the animal breathed room air spontanously. The carotid artery was cannulated for continuous measurement of arterial pressure. A polygraph (RM6000, Nihon Kohden, Japan) was used for simultaneous recording of BP, mean arterial pressure (MAP), and HR. During the experiment, the rectal temperature was detected and kept at 37 – 38 ℃.

**Brain microinjection** The animal was placed on a stereotaxic frame (SN-3, Narishige, Japan). After scalp incision and burr hole were made in the skull, according to the atlas of Paxinos and Watson[16], a stainless steel tube (outside diameter 0.20 mm) was unilaterally inserted into the AHA (1.30 – 1.80 mm behind the bregma, 0.5 – 1.0 mm left or right to the sagittal suture and 8.0 – 9.0 mm depth from the skull surface). The drugs were applied with a microinjector in an amount of 0.1 μL at a constant rate within 1 min.

**Drugs** Melatonin, prazosin, and luzindole were obtained from Sigma Chemical Company (USA) and were dissolved in 0.1 % ethanol saline. The values of pH of the above solutions were 7.0.

**Identification of brain sites for microinjection** At the end of each experiment, the sites of microinjection were marked by pontamine sky blue. After the brain had been fixed with 10 % formalin for 7 d, frozen cross sections (40 μm) were made. The actual sites of microinjection were identified according to Paxinos and Watson[16] (Fig 1).

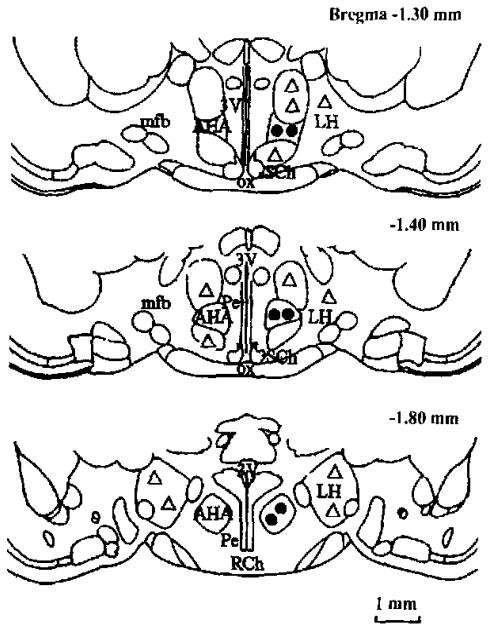


**Fig 1. The location of the microinjection of drugs (melatonin, its receptor antagonists, and vehicle of 0.1 % ethanol). The black circles and open triangles indicate the effective and ineffective sites respectively. 3V: 3rd ventrical; AHA: anterior hypothalamic area; LH: lateral hypothalamic area; mfb: medial forebrain bundle; ox: optic chiasm; Pe: periventricular hypothalamus nucleus; RCh: retrochiasmatic area; SCh: suprachiasmatic nucleus.**

**Statistical analysis** All data were expressed as $\bar{x} \pm s$, $n$ represents the number of rats. Statistical analysis was performed with ANOVA (analysis of variance). Two-way ANOVA was used for comparison of the effect before and after administration of drugs in different time, and one-way ANOVA was used for comparison of the effect between the groups with different drugs and their corresponding control groups. When the results were significant, Dunnett's test was performed to assess the significance of the differences.

## RESULTS

**Effects of microinjection of melatonin into the AHA on MAP and HR** Melatonin was microinjected into the AHA in normotensive (the sham stress) rats and stress-induced hypertensive rats, respectively. In normotensive rats, administration of melatonin at different concentrations caused depressive response immediately but to different extents (Fig 2). Melatonin 0.1 mmol/L decreased MAP from the control level of (13.2 ± 0.7) kPa to its minimum level of (11.9 ± 0.7) kPa ($P < 0.01$) and lasted about 15 min, while at the concentration of melatonin 1.0 mmol/L, MAP decreased from the control level of (13.5 ± 0.6) kPa to its minimum level of (11.4 ± 0.5) kPa ($P < 0.01$) and lasted about 20 min. The changes in HR were not significant.

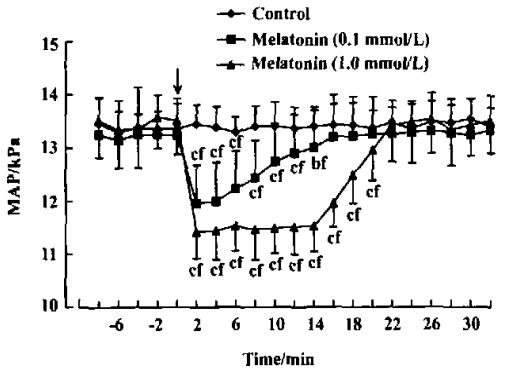


**Fig 2. Effects of microinjection of 0.1 % ethanol ($n$ = 6), melatonin 0.1 mmol/L ($n$ = 7) or 1.0 mmol/L ($n$ = 6) into the AHA on MAP in normotensive rats. The downward arrow indicates the start of the microinjection. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* the baseline before administration. $^fP < 0.01$ *vs* control.**

In stress-induced hypertensive rats, melatonin at a concentration of 1.0 mmol/L ($n$ = 6) decreased MAP from the control level of (16.7 ± 0.7) kPa to the level of (13.9 ± 0.7) kPa ($P < 0.01$) and lasted about 30 min (Fig 3). The changes in HR were not significant.

As a control, after 0.1 % ethanol ($n$ = 6) was microinjected into the AHA, there was no significant change on MAP and HR ($P > 0.05$). Nevertheless, there were significant differences in the above cardiovascular activities between groups of melatonin administration and the group of ethanol administration ($P < 0.01$) (Fig 2 and 3).

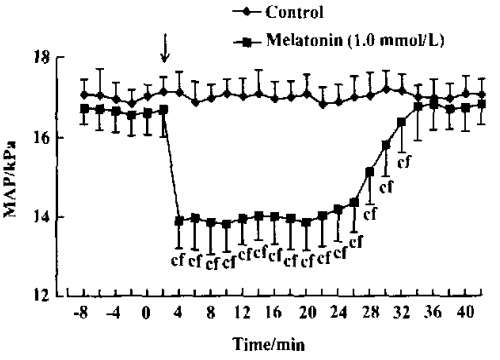


**Fig 3. Effects of microinjection of 0.1 % ethanol, melatonin 1.0 mmol/L into the AHA on MAP in srtess-induced hypertensive rats. The downward arrow indicates the start of the microinjection. $n$ = 5. $\bar{x} \pm s$. $^cP < 0.01$ *vs* the baseline before administration. $^fP < 0.01$ *vs* control.**

**Effects of prazosin microinjected into the AHA on the depressive response of melatonin** Effect of prazosin, an $ML_2$ receptor antagonist, on the melatonin-induced depressive responses was tested in normotensive rats. No significant changes in MAP and HR were seen in the group of rats treated with prazosin (10 mmol/L). In the other group, at 10 min after administration of prazosin (10 mmol/L), melatonin (0.1 mmol/L) was microinjected into the same site. With the presence of prazosin, melatonin retained its ability to reduce MAP to the same extent as observed in the group of rats treated with melatonin alone. It was reduced from its control level of (13.2 ± 0.8) kPa to its minimum level of (11.8 ± 0.8) kPa ($P < 0.01$) and lasted about 15 min (Fig 4), the changes in HR were not significant.

**Effects of luzindole microinjected into the AHA on the depressive response of melatonin** Effect of luzindole, competitive antagonist of melatonin $ML_1$ receptor, on the melatonin-induced depressive response was tested in normotensive rats. In rats treated with luzindole (10 mmol/L), no significant alteration in either MAP or HR was observed ($P > 0.05$). In the other group, at 10 min after administration of luzindole at the same concerctration, melatonin (0.1 mmol/L) was microinjected into the same site, decreases in MAP were not observed. Pretreatment with luzindole into the AHA almost completely prevented the depressive responses induced by following injection of melatonin 0.1 mmol/L ($P > 0.05$) (Fig 5), the changes in HR were not significant.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Nov; 22 (11)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

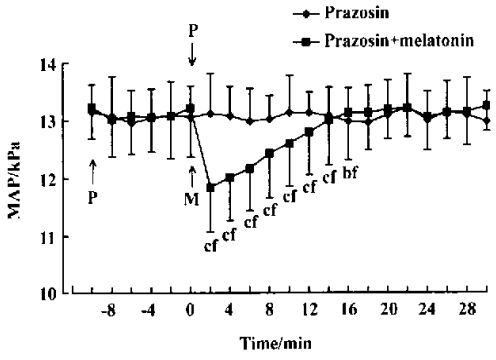


**Fig 4. Effects of microinjection of prazosin (10 mmol/L) and melatonin (0.1 mmol/L) with the pretreatment of prazosin into the AHA on MAP in normotensive rats. The downward arrow indicates the start of the microinjection of prazosin (P) in the control group, the upward arrows indicate the start of the microinjection of prazosin (P) and melatonin (M), respectively, in the group of melatonin admininstration. $n = 7$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* the baseline before administration. $^fP < 0.01$ *vs* control.**

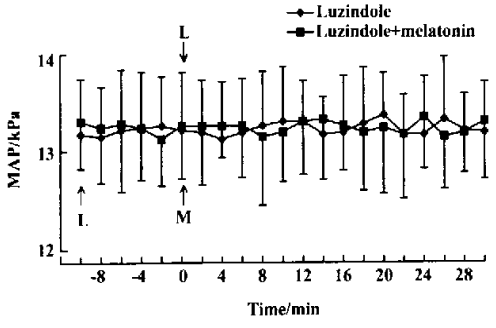


**Fig 5. The effects of microinjection of luzindole (10 mmol/L) and melatonin (0.1 mmol/L) with pretreatment of luzindole into the AHA on MAP in normotensive rats. The downward arrow indicates the start of the microinjection of luzindole (L) in the group of control. The upward arrows indicate the start of the microinjection of luzindole (L) and melatonin (M) in the group of melatonin administration. $n = 6$. $\bar{x} \pm s$.**

## DISCUSSION

The present study showed that microinjection of melatonin into the AHA produced a fall in MAP in rats with urethane and chloralose anesthesia, administration of an antagonist, prazosin, could not antagonize the depressive responses induced by melatonin, while luzindole could almost completely prevent the depressive responses induced by injection of melatonin. The results suggest that melatonin, one of the main pineal hormones, participates in the regulation of cardiovascular function as a hypotensive factor in the CNS.

Indeed, several laboratories have reported that male SD rats develop hypertension following pinealectomy[2]. Pinealectomy-induced hypertension can be prevented by oxypertine, a certainly acting adrenergic blockade with a 5-methoxyindole structure resembling melatonin[14]. In addition, melatonin was shown to reduce blood pressure in human beings[6] and in intact[10], pinealectomized rats[17], and SHR[7]. Furthermore, it was found that melatonin concentration in the nocturnal serum of SHR was elevated at the prehypertensive stage, while it decreased after the development of hypertension when compared with age-matched normotensive Wistar-Kyoto (WKY) rats[5]. Some more recent reports showed that oral administration of melatonin (1 mg) was able to reduce blood pressure, influence artery blood flow, and blunt noradrenergic activation in healthy men[18] and women[19]. It indicates that melatonin may blunt the cardiovascular activities and may have both physiopathologic and clinical implications. These observations support the possibility that the pineal gland may play a role in cardiovascular control and that an abnormality in melatonin secretion might produce hypertension.

In fact, it has been proposed that melatonin may act as an endogenous hypotensive factor, possibly by stimulating central inhibitory adrenergic pathways[14]. After pinealectomy adrenal steroid level increased[2]. Indeed, melatonin-induced hypotension was abolished by pretreatment with spinal transaction, while melatonin-induced bradycardia was abolished by pretreatment with bilateral vagotomy in rats under general anesthesia. It is also demonstrated that melatonin decreases brain (AHA and corpus striatum) serotonin release and results in a decrease in sympathetic efferent activities or an increase in vagal tone which leads to a decrease in arterial pressure in rats[10].

The AHA of rat brain is a site of central cardiovascular regulation functioning as an integrative center[20]. [2-$^{125}$I]iodomelatonin binding sites were found over the AHA[13]. In the current results, microinjection of melatonin into the AHA caused a decrease in MAP. These results indicate that melatonin acts as a hypotensive factor and the AHA may be one of the important central areas where melatonin can exert modulatory effects on cardiovascular activities.

The mechanism of action of melatonin remains to be

unraveled. Receptors for melatonin are present in medial basal hypothalamus, and a number of hypothalamic metabolic functions and constituents are affected by melatonin treatment, including protein synthesis, serotonin and γ-aminobutyric acid content, neurotransmitter uptake and release, axonal transport, tubulin levels and prostagladin (PG) and neurohormone release. Measurement of cyclic neuleotide accumulation in rat medial hypothalamus incubated *in vitro* indicated that melatonin significantly increased cGMP and depressed cAMP levels. It has been reported that a general effect of melatonin in target tissues may be the increase of cGMP levels[21]. The linkage between cyclic nucleotide changes and the depressive response by melatonin reported herein deserves further investigation.

Prazosin, a known $\alpha_1$-adrenoceptor antagonist that also has a high affinity for $ML_2$ receptor[22], had not any central effect on blood pressure in the present study, and it could not antagonize the depressive response induced by melatonin.

Luzindole (2-benzyl-*N*-acetyltrytamine), an *N*-acetyltrytamine lacking the 5-methoxy group and possessing a 2-benzyl substitution, has been shown to be the competitive melatonin $ML_1$ receptor antagonist[22,23]. It has been reported that this competitive melatonin antagonist is effective in blocking melatonin receptors in brain and is active *in vivo*[23]. In the current study, luzindole was able to almost completely prevent the depressive responses induced by following injection of melatonin. These results suggest that in the brain, luzindole blocks the activation of melatonin receptor by melatonin.

Altogether, these observations suggest that melatonin can inhibit the cardiovascular activity and the effects are mainly due to activation of $ML_1$ receptors in the brain of rats.

**ACKNOWLEDGEMENTS** The authors wish to thank QIAN Yuan and CHEN Zhi-Jie for their help with the use of electric instrument in this experiment.

## REFERENCES

1 Cagnacci A. Melatonin in relation to physiology in adult humans. J Pineal Res 1996; 21: 200 – 13.
2 Zanoboni A, Zanoboni-Muciaccia W. Experimental hypertension in pinealectomized rats. Life Sci 1967; 6: 2327 – 31.
3 Karppanen H, Vapaatalo H, Lahovarra S, Paasonen MK. Studies with pinealectomized rats. Pharmacology 1970; 3: 76 – 84.
4 Birau N. Melatonin in human serum: progress in screening investigation and clinic. In: Birau N, Shloot W, editors. Melatonin: current status and perspectives. Oxford: Pergamon Press; 1981. p 297 – 326.
5 Kwashima K, Nagakura A, Wurzburger RJ, Specter S. Melatonin in serum and the pineal of spontaneously hypertensive rats. Clin Exp Hypertens (A) 1984; 6: 1517 – 28.
6 Birau N, Peterssen U, Meyer C, Gottschalk J. Hypotensive effect of melatonin in essential hypertension. IRCS Med Sci Surg Transplant 1981; 9: 906.
7 Kawashima K, Miwa Y, Fujimoto K, Oohata H, Nishino H, Koike H. Antihypertensive action of melatonin in the spontaneously hypertensive rat. Clin Exp Hypertens (A) 1987; 9: 1121 – 31.
8 Tan DX, Manchester LC, Reiter RJ, Qi W, Kim SJ, El-Sokkary G. Ischemia/reperfusion-induced arrhythmias in the isolated rat heart: prevention by melatonin. J Pineal Res 1998; 25: 184 – 91.
9 Wang H, Ng YB. Hypotensive activity of the pineal indoleamine hormones melatonin, 5-methoxytryptophol and 5-methoxytryptamine. Pharmacol Toxicol 2000; 86: 125 – 8.
10 Chuang JI, Chen SS, Lin MT. Melatonin decrease brain serotonin release, arterial pressure and heart rate in rats. Pharmacology 1993; 47: 91 – 7.
11 Capsoni S, Stankov BM, Fraschini F. Reduction of regional cerebral blood flow by melatonin in young rats. Neuroreport 1995; 6: 1346 – 8.
12 Viswanathan M, Laitinen JT, Saavedra JM. Expression of melatonin receptors in arteries involved in thermoregulation. Proc Natl Acad Sci USA 1990; 87: 6200 – 3.
13 Williams LM, Hannah LT, Hastings MH, Maywood ES. Melatonin receptors in the rat brain and pituitary. J Pineal Res 1995; 19: 173 – 7.
14 Karppanen H, Airaksinen MM, Sarkimaki I. Effect in rats of pinealectomy and oxypertine on spontaneous locomotor activity and blood pressure during various light schedule. Ann Med Exp Biol Fenn 1973; 51: 93 – 103.
15 Berria M, DeSantis M, Mead RA. Lesions to the anterior hypothalamus prevent the melatonin-induced lengthening of delayed implantation. Endocrinology 1989; 125: 2897 – 904.
16 Paxinos G, Watson C. The rat brain in sterotaxic coordinates. 2nd ed. New York: Academic Press; 1986.
17 Holmes SW, Sugden D. The effect of melatonin on pinealectomy-induced hypertension in the rat. Br J Pharmacol 1976; 56: 360 – 1.
18 Arangino S, Cagnacci A, Angiolucci M, Vacca AM, Longu G, Volpe A, *et al*. Effects of melatonin on vascular reactivity, catecholamine levels, and blood pressure in healthy men. Am J Cardiol 1999; 83: 1417 – 9.
19 Cagnacci A, Arangino S, Angiolucci M, Maschio E, Melis GB. Influences of melatonin administration on the circulation of women. Am J Physiol 1998; 274: R335 – R338.
20 Abboud FM. The sympathetic nervous system in hyperten-

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Nov; 22 (11)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

sion. Clin Exp Hypertens (A) 1984; 6: 43 – 60.
21 Vacas MI, Keller Sarmiento MI, Cardinali DP. Melatonin increases cGMP and decreases cAMP levels in rat medial basal hypothalamus *in vitro*. Brain Res 1981; 225: 207 – 11.
22 Dubocovich ML. Melatonin receptors: are there mutiple subtypes? Trends Pharmacol Sci 1995 16: 50 – 6.
23 Dubocovich ML. Pharmacology and function of melatonin receptors. FASEB J 1988; 2: 2765 – 73.

## 下丘脑前核微量注射褪黑素及其受体拮抗剂对大鼠血压和心率的影响[1]

丁超南，曹银祥，周 莉，朱大年[2]，沈仲元[3]，费民毅[3]，余 平[3]（复旦大学医学院生理教研室，上海 200032；[3]上海中医药大学，上海 200032，中国）

**关键词** 褪黑激素；下丘脑前核；血压；心率

**目的**：研究下丘脑前核微量注射褪黑素及其受体拮抗剂对正常血压和应激性高血压大鼠心血管活动的影响. **方法**：微量注射褪黑素及其受体拮抗剂至下丘脑前区前核，记录血压、平均动脉压和心率. **结果**：微量注射褪黑素可降低平均动脉压，其竞争性 $ML_1$ 受体拮抗剂 luzindole 可完全阻断褪黑素的降压反应，而其 $ML_2$ 受体拮抗剂 prazosin 不能阻断褪黑素的降压反应. **结论**：褪黑素为一种降压因子，其降压反应主要通过激活 $ML_1$ 受体，而不是 $ML_2$ 受体来介导的，下丘脑前核是褪黑素影响心血管活动的重要中枢部位.

（责任编辑 吴民淑）