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The Achilles Heel in Melatonin: Asthma

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ABSTRACT

Asthma is a clinical syndrome characterized by chronic airway inflammation, airway responsiveness, and expiratory airflow limitation. Nocturnal symptoms and decreases in lung function are common aspects of the asthma clinical syndrome. Nocturnal symptoms also appear to be associated with asthma-related mortality.

In addition to its importance to the regulation of human circadian rhythms, an accumulating body of evidence also suggests that melatonin is also involved in the regulation of smooth muscle tone. For this reason, this study aimed to evaluate contraction and relaxation responses in tracheal smooth muscle rings obtained from rats treated with melatonin.

Following administration of melatonin (50mg/kg/day) at the same time every day for 6 weeks, *in vitro* organ bath experiments were performed with rat tracheal preparations exposed to contractile (acetycholine and serotonin) and relaxant (theophylline and papaverine) agents. Melatonin treatment strengthened contraction responses, but did not affect relaxation responses in rat tracheal preparations. We think that melatonin might play a role in the pathogenesis of nocturnal asthma.

Therefore, clinicians should be aware of the importance of melatonin to nocturnal exacerbation of asthma symptoms and alert asthmatic patients that use exogenous melatonin supplementation of its potential negative effects.

Key words: Bronchial asthma; Circadian rhythm; Melatonin; Trachea

INTRODUCTION

Bronchial asthma (BA) is characterized by chronic airway inflammation and reversible airflow limitation.[1] The symptoms of BA include dyspnea, wheezy chest, and croupy cough.[2] In most asthmatic patients these symptoms become more severe between midnight and early morning.[3,4] Thus, successful asthma management must address the major features of the disease and chronopharmacotherapy should be included in the treatment of BA because of the nocturnal exacerbation of symptoms.

Melatonin is the primary hormone secreted by the pineal gland and is thought to play a central role in the biological regulation of circadian rhythms; therefore,

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melatonin might also play a role in nocturnal asthma.[5] Although, melatonin has been shown to exacerbate allergic airway inflammation and increase airway smooth muscle tone, it might have a negative effect on the severity of asthma.[6,7] Furthermore, melatonin receptors have been isolated in lung tissue.[8] As it is known that melatonin levels are higher in patients with nocturnal asthma than in patients with non-nocturnal asthma and healthy controls, the possibility that nocturnal exacerbation of asthma may be due to changes in the level of circulating melatonin and the kinetics of melatonin–receptor interactions in asthma cannot be excluded.[9]

We hypothesized that melatonin might play an important role in the regulation of nocturnal asthma by altering airway smooth muscle responsiveness. To test this hypothesis, the present *in vitro* experimental study used tracheal preparations obtained from rats treated with melatonin and those not treated with melatonin to investigate responses to some contractile and relaxant agents. To the best of our knowledge, this is the first study to examine the effect of melatonin on the isolated rat trachea.

MATERIALS AND METHODS

Animal and Tissue Preparations

Akdeniz University, School of Medicine, Animal Use and Care Committee approved the study protocol. In all, 30 male Wistar rats were divided into 2 groups: control and melatonin treatment. Animals were housed in light (12 h light-dark cycle) and temperature (20ºC)-controlled rooms, and had free access to water and food. Melatonin was dissolved in ethanol (1 mg /ml) and administered intraperitoneally (50 mg/kg/day) for 6 weeks. Concurrently, its vehicle was administered to the control group according to the same protocol.

After 42 days of treatment, the rats were stunned and decapitated. The trachea was removed rapidly and then 3-mm rings were cut and mounted in thermostatically controlled (37ºC) organ baths that contained 20 mL Krebs-Henseleit solution (KHS), consisting of the following (in mM): NaCl 118, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1.0, $MgSO_4$ 1.2, $CaCl_2$ 2.5 and glucose 11.2. The solution's pH was maintained at 7.4 during aeration with 95% O_2 and 5% CO_2. The rings were suspended under a tension of 0.75 g, which was determined to be the optimal preload in previous studies and during our preliminary experiments.[10] The preparations were equilibrated under this tension for 1 h and washed with KHS every 15 min before begining the experiments. Isometric tension was continously measured using a BIOPAC FDT-05 force displacement transducer (BIOPAC Systems, Goleta, GA, USA) and isometric changes were recorded using a BIOPAC MP35 Transducer Data Acquisition System.

Isolated Tracheal Ring Experiments

Isolated tracheal rings were used in each experiment to measure contraction and relaxation responses to different types of contractile [acetylcholine (Ach) and serotonin (5-hydroxytryptamine, 5-HT)] and relaxant (theophylline and papaverine) agents. Dose-response curves to Ach (10^{-9}-10^{-4}M), 5-HT (10^{-9}-10^{-5}M), theophylline (10^{-9}-10^{-4}M), and papaverine (10^{-8}-10^{-4}M) were made cumulatively. In the experiments performed with contractile agents the rings were exposed to the lowest concentration of Ach or 5-HT until the maximal response was observed, and then the rings were exposed to the next highest concentration. To examine the relaxant effects of theophylline (10^{-9}-10^{-4}M) or papaverine(10^{-8}-10^{-4}M), the rings were first contracted with a submaximal concentration of Ach (10^{-6}M).

Drugs and Preparation of Drug Solutions

The following drugs were obtained from the Sigma Chemical Company (St. Louis, MO, USA): Ach, 5-HT, melatonin, theophylline and papaverine. Ach, papaverine and 5-HT were dissolved in distilled water. Ethanol was used to dissolve theophylline and melatonin. Ethanol was tested alone and had no effect on the tracheal preparations. Drug solutions were prepared fresh the day they were used.

Statisitical Analysis

All results were expressed as the mean ± SD. Contractile effects are expressed as a percentage of the contraction elicited by 80 mM KCl solution which is taken as 100 % in each tissue. Relaxation responses to theophylline or papaverine were expressed as a percentage of the Ach–induced (10^{-6}M) contraction. E_{max} (the maximal response) and EC_{50} (the concentration producing 50% of the maximal response) were determined via non-linear regression analysis (sigmoidal dose-response with variable slope) using GraphPad Prism, version 5.01 (GraphPad Software Inc., San Diego, CA, USA). Sensivity/ potency is expressed as pD_2= -log EC_{50}. Data were analyzed by two-way ANOVA for multiple comparisons followed by Bonferroni post-hoc test. The level of statistical significance was set at $p<0.05$.

RESULTS

Melatonin and ethanol (as avehicle) were administered to melatonin-treatment and control groups, respectively. Administration protocol was done intraperitoneally (50mg/kg/day) at the same time every day for 6 weeks. We did not observe any significant changes in rats' metabolism or behaviour. Tracheas isolated from the melatonin treatment group exhibited higher sensivity (pD2) and maximal contractility (Emax) to 5-HT or Ach than those in the control group. In contrast, there wasn't a difference in response to theophylline or papaverine between the rat tracheas from the melatonin treatment and control groups (Table 1).

Administration of 5-HT (10^{-9}-10^{-5}M) and Ach (10^{-9}-10^{-4}M) caused a concentration-dependent contraction response in the rats' tracheas. These contraction responses to 5-HT and Ach were significantly greater in the tracheal rings from melatonin treatment group than in those from control group (Figure 1). Both theophylline (10^{-9}-10^{-4}M) and papaverine (10^{-8}-10^{-4}M) caused concentration-dependent relaxation responses in rat tracheal rings precontracted using Ach (10^{-6}M); however, the responses to theophylline and papaverine did not significantly differ between melatonin treatment and control groups (Figure 2).

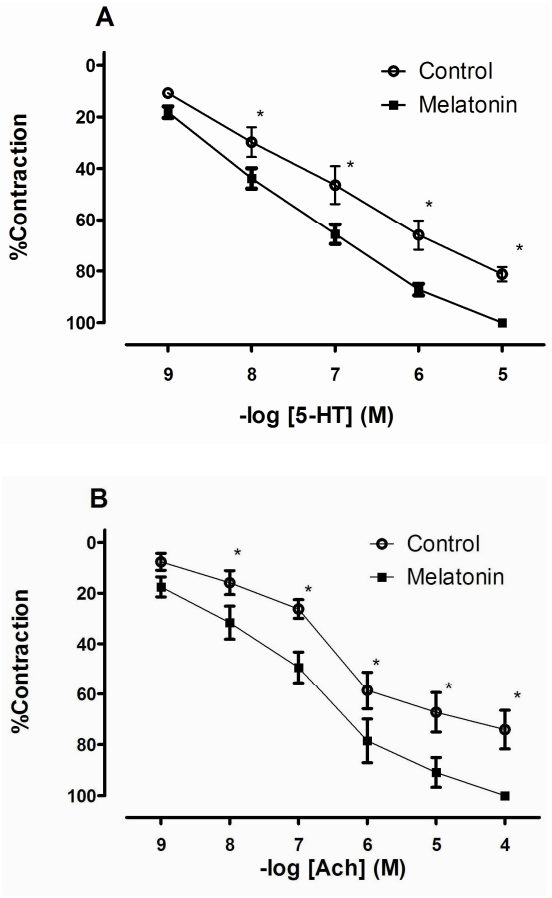

Figure 1. The contractile effects of 5-HT (A) and Ach (B) in isolated tracheal rings obtained from the melatonin treatment and control groups. Each point represents the mean ± SD, as shown by vertical bars. *p<0.05.

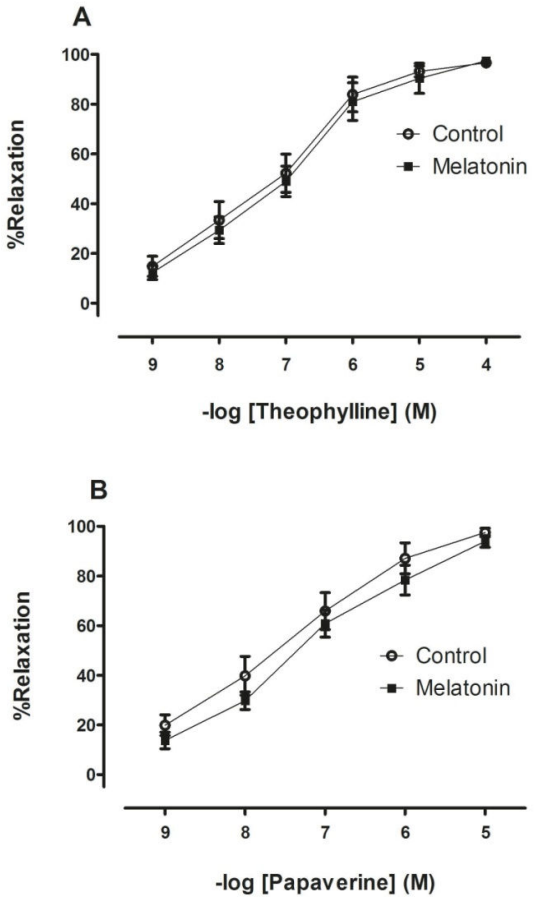

Figure 2. The relaxant effects of theophylline (A) and papaverine (B) on isolated tracheal rings obtained from the melatonin treatment and control groups and precontracted using Ach. Each point represents the mean ± SD, as shown by vertical bars. *p<0.05.

Table 1. pD_2 and E_{max} values for relaxant and contractile agents.

	Control		Melatonin-treated	
	pD_2	Emax	pD_2	Emax
Ach	6.33 ±0.02	74.8 ±2	6.91 ± 0.04*	98.3 ±5*
5-HT	6.56 ±0.03	80.8 ±7	7.47 ± 0.03*	95.5 ±6*
Theophylline	6.54 ±0.05	96.6 ±2	6.48 ± 0.04**	97.4 ±3**
Papaverine	7.37 ± 0.04	97.5±3	6.98 ± 0.05**	93.9 ±6**

*p<0.05 denotes significant from respective control values in contractile agent (Ach and 5-HT) responses.

**p<0.05 denotes significant from respective control values in relaxant agent (theophylline and papaverine) responses.

DISCUSSION

Nocturnal asthma is a common and potentially fatal complication of asthma, characterized by exacerbation of symptoms, need for medication, and/or deterioration of lung function, which typically occurs between 04:00 and 06:00 h.[11] This is more common, especially in patients with moderate or severe asthma. Nocturnal increase in bronchial resistance may be associated with several factors, including an elevated serum melatonin level.[9]

There is a lack of basic research on the effect of melatonin on tracheal relaxation and contraction responses. To the best of our knowledge only Bruderman et al. published results of a study on melatonin in isolated cat tracheal segments.[12] Ach and 5-HT are 2 most commonly used agonists for measuring contractile responses in tracheal smooth muscle.[13] In the present study contractile responses to both Ach and 5-HT were significantly greater in the tracheas obtained from the melatonin group, whereas relaxation in response to theophylline or papaverine were not significantly changed.

Reports of melatonin's involvement in various physiological processes and its affect on smooth muscle tone in different tissues are inconsistent. For instance, similar to its relaxant effects on Ach-induced contraction in isolated teleost fish intestine, melatonin also decreased contractions in the small and large intestines in rats, whereas it caused contraction of guinea pig colonic smooth muscle.[14-16] The most likely explanation for the differences in the reported effects of melatonin is differences in species studied, experimental conditions, and duration of melatonin administration (chronic or acute).

It is that the increase in contraction responses to Ach in the melatonin-treated rat tracheas observed in the present study might have been due to modulation of muscarinic receptors in airway smooth muscle and secretory glands in response to the chronic administration of melatonin. Interesting results regarding the interactions between melatonin and the cholinergic system have been published.[17-20] As mentioned previously, exogenous melatonin potentiates sympathetic neurotransmission in the prostatic portion of the rat vas deferens by increasing contraction responses via modulation of Ach receptor binding sites.[21] Almeida-Paula et al. reported that melatonin modulates the number of Ach receptors in the rat myotube.[19] Another possible explanation for the increase in contraction responses to Ach might be alteration in it's synthesis process, as Carneiro et al. briefly observed that regulation of protein synthesis might be one of the mechanisms by which melatonin modulates endogenous rhythms.[22] The present study's findings are in agreement with those of Gomez-Pinilla et al., who reported that treatment of aged animals with melatonin restored the contractile response of bladder smooth muscle as the result of increased Ca^{+2} sensivity.[23]

Serotonin leads to contraction of airway smooth muscle if released from mast cells in situ or if applied exogenously *in vitro* [24]. In the present study, 5-HT-induced contractions also increased in the melatonin treatment group. It has been reported that contractions evoked by 5-HT are largely dependent on the release of Ach.[25] Consistent with this assumption, the 5-HT-induced bronchoconstriction observed in the present study could be considered a part of cholinergic contraction and it is not surprising to observe similar potentialized contraction responses. On the other hand, melatonin potentiated contractile responses to serotonin by inhibiting the action of nitric oxide on coronary vascular smooth muscle; however, further investigations are needed to clarify the mechanism of action.[26-28]

Although it has been suggested that melatonin has relaxant effects in rat ileal smooth muscle and the rat urinary bladder, we observed that chronic melatonin administration did not significantly alter relaxant responses to theophylline or papaverine.[29,30] Theophylline and papaverine produced concentration-dependent relaxant responses in Ach-precontracted isolated tracheal preparations obtained from the melatonin treatment and control groups. Papaverine, a non-selective smooth muscle relaxant, exerts its inhibitory effects in the trachea via increasing cyclic adenosine monophosphate (cAMP) content or decreasing intracellular Ca^{+2}.[31] Furthermore, theophylline is used worldwide for the treatment of asthma, but its tracheal smooth muscle relaxation mechanism of action is not well understood. It is presumed that theophylline induces relaxation of tracheal smooth muscle via the action of cAMP and decreasing the level of intracellular Ca^{+2}, as does papaverine.[32] Melatonin (0.01 pg/mL) increased the opening of the receptor-operated channels for calcium and/or the Ca^{+2} mobilization from intracellular stores.[33] Accordingly, it is possible that intracellular Ca^{+2} regulation might be one of the pathways by which melatonin exerts its effects. This sentence was checked and corrected as follows:

As there was a difference only in responses to contractile agents in our study, it is concluded that the observed increase in contractile responses and unchanged relaxation responses may have been due to the direct contractile effect of melatonin.

Findings concerning the level of melatonin in asthmatic patients are inconsistent. Recently, Gumral et al. reported that serum melatonin levels in patients with BA were significantly lower than those in healthy controls.[34] It is known that melatonin is released rapidly and due to its high lipophilicity it easily crosses cell membranes. Moreover, melatonin is not stored long term in the pineal gland, and 6-sulphatoxymelatonin (MT6) is the major melatonin metabolite in humans, which is used as an indicator of endogenous melatonin concentrations in plasma.[35,36] Therefore, MT6 should be used as an indicator of endogenous melatonin in that study.

To the best of our knowledge the present study is the first to investigate the effect of long-term (6 weeks) melatonin administration on tracheal smooth muscle in the rat. Commonly, experiments with rodents conducted to observe acute *in vitro* effects of melatonin are performed in organ baths.[37,38] We believe that duration of administration is important for evaluating the long-terms effects and useful for making a conclusion on drug safety. In the present study unusual behavior, changes in body weight, and death did not occur in either group. Based on the present findings, we think that melatonin is both a potent antioxidant and vasorelaxant, but in some experiments it may behave as a pro-oxidant and vasoconstrictor.[39-44] Thus, additional research and clinical trials are needed to more clearly understand the effects of melatonin.

CONCLUSION

Additional research is needed to fully understand the clinical implications of the present study's results. The present results indicate that melatonin caused an increase in contraction responses in the isolated rat trachea, a finding that may have clinical relevance in asthma patients that use over-the-counter pharmaceutical preparations containing melatonin. In particular, patients with severe asthma should avoid using melatonin supplements until further research elucidates the clinical effect of melatonin on the symptoms of asthma.

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