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Investigation the effect of ramelteon on urinary bladder smoth muscle contraction-relaxation mechanism

Zubeyde Ercan[a,*], Munevver Gizem Hekim[b], Gokhan Zorlu[c], Ozgur Bulmus[d], Abdullah Yasar[e], Ihsan Serhatlioglu[c], Emine Kacar[b]

[a] *Firat University, Faculty of Health Sciences, Department of Physiotheraphy and Rehabilitation, Elazig, Turkey*
[b] *Firat University, Faculty of Medicine, Department of Physiology, Elazig, Turkey*
[c] *Firat University, Faculty of Medicine, Department of Biopysics, Elazig, Turkey*
[d] *Balikesir University, Faculty of Medicine, Department of Physiology, Balikesir, Turkey*
[e] *Firat University, Vocational School of Health Services, Elazig, Turkey*

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Abstract

Aim: A melatonin receptor agonist ramelteon is a drug that is also used in urinary tract infections. Melatonin is known to have an effect on smooth muscle contraction. In this study, we aimed to question whether ramelteon exerts a melatonin-like effect on bladder contractions.

Material and Methods: Wistar-albino (n = 8) intact female rats were used in the study. After decapitation, 1.5x5 mm bladder strips were examined in an isolated organ bath in which there was Krebs-Henseleit solution. After the regulation period, ramelteon was applied non-cumulatively in two separate doses of 0.2µM, 1µM. The area under the curve (AUC) and peak to peak (p-p) values before and after the application were normalized as % change.

Results: Exposure of bladder strips to ramelteon significantly increased contractions. Ramelteon caused a statistically significant increase in p-p, AUC values of spontaneous bladder contractions at a dose of 0.2 and 1µM ($p < 0.05$).

Conclusion: Ramelteon has an activator effect on spontaneous bladder contractions unlike melatonin. The physiopathological mechanisms under activator activity need to be investigated with further studies. This effect should be taken into account in clinical use.

Introduction

Ramelteon, a melatonin receptor agonist, is a drug with a new mechanism of action that adjusts the sleep-wake rhythm without producing sedative effects and induces sleep [1, 2]. It is a preferred agent for insomnias with difficulty falling asleep or with circadian rhythm disturbances [3]. Ramelteon is structurally different from melatonin and has been shown to be more potent up to 17 times at certain melatonin receptors [4]. It is subject to good manufacturing practices enforced by the United States Food and Drug Administration, and there is scientific evidence of safety and efficacy at certain doses [3, 5]. Melatonin has been shown to inhibition in different organ smooth muscles tonus and has very strong antioxidative effects. [6, 7]. Melatonin increases bladder capacity and decreases urine volume through its action on the central nervous system [8], and also relaxes increased urinary bladder contractions with effects on peripheral nervous system [9]. The effects of ramelteon, on bladder smooth muscle are unknown. Studies have shown that ramelteon is an safe and effective treatment method in patients with nocturia and insomnia [10]. It has been shown that ramelteon, which is used to increase the bladder capacity at night in patients with insomnia caused by nocturia, reduces the frequency of nocturnal voiding and improves sleep disorders [11). In this study, it was reported that the use of ramelteon resulted in improvement in both general lower urinary tract infections and nocturia. Since ramelteon does not cause drowsiness in older populations with insomnia, it has the potential to reduce the risk of falls in patients who wake up at night to urinate [12]. However, sleep problems due to disturbances in body rhythm caused by working conditions such as irregular shifts have been reported to cause inflammation [13]. It is known that sleep disorders and lower urinary tract symptoms (LUTS) are closely related. The prevalence of these two conditions increases in the older population, and high prevalence of sleep disorders

*Corresponding author:
Email address: zubeydeercan@firat.edu.tr (Zubeyde Ercan)

are associated with a high LUTS scores [14]. Although ramelteon has an anti-inflammatory effect at the systemic level, it has been shown to have beneficial effects in improving sleep disorders and LUTS. [15]. However, the effects of this agent on the bladder smooth muscle contraction-relaxation mechanism are unknown. There are no studies investigating the effectiveness of the ramelteon, which is effective in lower urinary tract infections, on bladder contraction. Therefore, we aimed to examine the effect of ramelteon on bladder contractions in our study.

Materials and Methods

Animals

All experimental procedures were performed on 8 female Wistar rats (200-230 g) and these animals were obtained from Fırat University Experimental Animal Unit. The experimental protocols were approved by Firat University Local Ethical Committe for the Experimental Animal Research. Rats were housed in a controlled environment at 12 hour light-dark cycle, 21 ± 2 °C temperature, 50-60% relative humidity. Access of animals to feed and water was provided ad libitum.

Preparation of Tissues

The pelvic cavities of the decapitated female wistar rats were opened rapidly. Bladder tissues were removed and separated from the ureters. The urinary bladder was cut into uniform longitudinal strips (1.5x5 mm) for use in organ bath. The sections were submerged into Krebs-Henseleit solution (1.18 mM KH_2PO_4, 24.7mM KCl, 1.2 mM $MgSO_4$, 118mM NaCl, 15.8 mM $NaHCO_3$ 0.4 mM $CaCl_2$, 11.5 mM glucose, 0.016 mM EDTA).

In Vitro Contractility Assay in Organ Bath

Bladder sections were then suspended by means of silk threads in a 5 ml organ bath (MAY IOBS 99) containing Krebs solution oxygenated with a mixture of carbon dioxide and oxygen (5: 95%) and at a constant temperature of 37°C. The lower end of the sections was fixed on a metal hook, and the upper end was attached to an isometric power converter; transducer signals were amplified and recorded in the recording unit. The sections under a passive resting tension of 1.5 g for 90 minutes were allowed to equilibrate during which rhytmic and spontaneus contractions developed. During the equilibration period the bath solution was replaced with a new one every 15 minutes. After the equilibration period, ramelteon was applied non-cumulatively in two separate doses of 0.2 μM, 1 μM on sections that showed spontaneous contractions. The effects of ramelteon on spontaneous bladder contractions were measured by changes in the mean peak to peak (p-p) and area under the contraction curve (AUC). Contractile activity (mean p-p and AUC) was taken as 100% during the control period. AUC, p-p values before and after administration were normalized as% change.

Statistical Analysis

Statistical analysis of the data was evaluated using the Paired T test in the SPSS 22.0 program. All values were determined as mean ± standard deviation (mean ± SD). For all analysis, $p < 0.05$ was statistically significant.

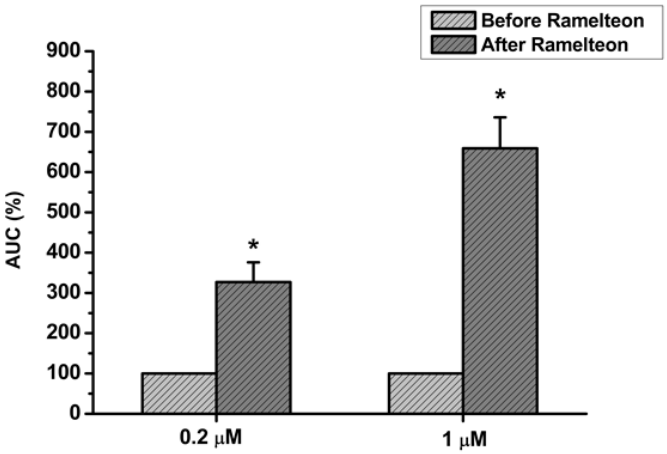

Figure 1. Effects of ramelteon on bladder contractions as area under the curve (AUC) measurements. * $p < 0.05$.

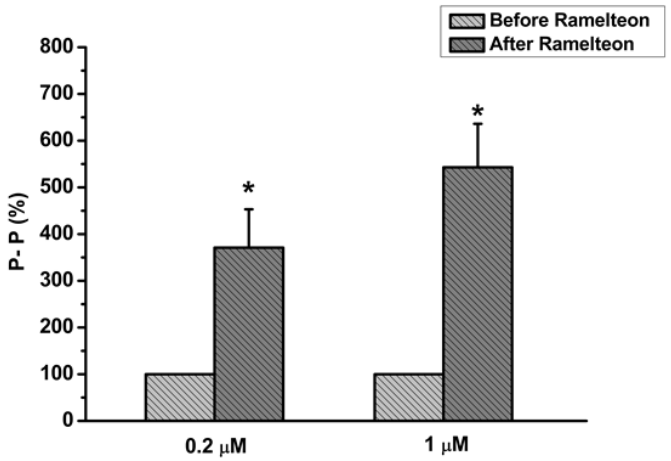

Figure 2. Effects of ramelteon on bladder contractions as peak to peak (p-p) measurements. * $p < 0.05$.

Results

Ramelteon caused a statistically significant increase in AUC and p-p values of spontaneous bladder contractions at doses of 0.2 μM and 1 μM (Figure 1 and Figure 2). This effect was observed significantly 5 minutes after the application. After the 0.2 μM dose was applied, the p-p values of the contractions were calculated as 371.1 ± 218 and the AUC values as 327.9 ± 131.9. After ramelteon applied at concentrations of 1 μM, the p-p values of contractions were calculated as 543.8 ± 248.1 and AUC values as 659 ± 204.5. It was observed that p-p ($p = 0.017$ and $p = 0.003$, respectively) and AUC values of ramelteon applied at concentrations of 0.2 μM and 1 μM significantly increased compared to the pre-application values ($p = 0.004$ and $p = 0.001$, respectively). The original traces obtained in the isolated organ bath at 0.2 μM, 1μM doses were shown in Figure 3 and Figure 4, respectively.

Discussion

Ramelteon caused a statistically significant increase in spontaneous bladder contractions as demonstrated in p-p and AUC values. Ramelteon, a melatonin receptor agonist, induced spontaneous contractions in the urinary blad-

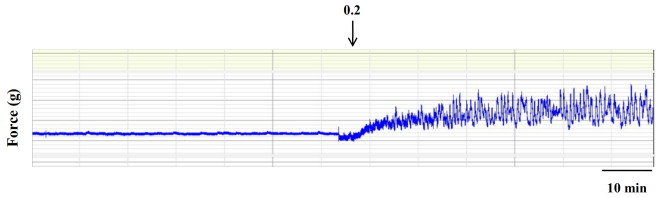

Figure 3. Original trace obtained when a 0.2 μM dose of ramelteon was administered in an isolated organ bath.

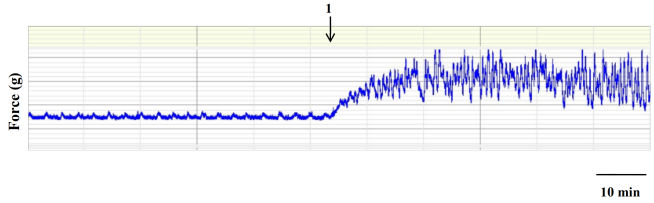

Figure 4. Original trace obtained when a 1 μM dose of ramelteon was administered in an isolated organ bath.

der tissue. It was seen that this effect was greater as the dose increased. The effects of melatonin on nocturia are known [16, 17]. In a study in rats, melatonin was found to increase bladder capacity via the GABAergic system [8]. However, ramelteon, a selective melatonin receptor-2 (MT-2) and MT1 melatonin receptors agonist, has been shown to not affect the GABAergic system. Ramelteon is a drug that adjusts the onset of sleep to the correct time (ie, normalizes the body clock) without increasing melatonin secretion, and its affinity for its receptors (MT1 and MT2) is higher than melatonin [18]. In studies conducted with patients with chronic insomnia problems, it has been shown that ramelteon is effective in reducing sleep latency and does not cause any significant clinical side effects. It has also been shown to increase overall sleep duration and sleep efficiency [19]. Melatonin is a hormone of pineal gland that is predominantly secreted at night. Nighttime melatonin production is impaired in elderly people, and several clinical studies have shown that administering melatonin exogenously to these people improves sleep problems [20, 21] and nighttime micturition [16, 17]. It is the main physiological determinant of circadian rhythm and shows the physiological decrease in nocturnal urine output and frequency in healthy individuals through its direct or indirect effects on other hormones [22]. Melatonin has also been reported to affect tone of smooth muscle. It has been shown to reduce small and large intestine smooth muscle contraction in rats [23], however, it has been shown to induce colonic smooth muscle contraction in guinea pigs [24]. Melatonin caused vasoconstriction in rat caudal and cerebral arteries in a dose-dependent manner [25, 26], but inhibit norepinephrine-induced vasoconstriction [27] and induce relaxation of arterial smooth muscle of porcine [28]. Melatonin has a regulatory effect on smooth muscle motility of gastrointestinal system and has been reported to have both contractile and relaxing effects [9, 29]. In addition, it inhibits oxytocin-induced and spontaneous contractions in the myometrium of pregnant and non-pregnant rats by inhibiting calcium channels [6]. It inhibits the contraction of bladder induced by acetylcholine and potassium chloride [9]. Melatonin has been shown to inhibit potassium chloride and acetylcholine-induced contractions when applied exogenously to bladder strips from guinea pigs [9]. In a cystometric study performed in Sprague Dawley female rats, melatonin was shown to significantly increase bladder capacity and improve detrusor over activity in a dose-dependent manner [8]. In a study performed in patient with bladder outlet obstruction due to benign prostatic enlargement, melatonin showed a significant improvement in nocturia frequency and nocturia-related discomfort without any significant side effects [16]. In a randomized controlled study, administration of melatonin resulted in a decrease in nighttime urination frequency and an improvement in quality of life scores in individuals with nocturia [17]. It has been shown that the combined use of melatonin and low-dose trospium has a strong inhibitory capacity in vitro on the contraction of bladder strips in rat [30]. Contrary to all these findings, we found that ramelteon, exerts an activator effect on bladder contractions. This finding is the opposite of melatonin's inhibitory effect on bladder contractions. This suggests that ramelteon acts in a way other than melatonergic effect on bladder contraction. In the later stages of our study, we aim to elucidate the physiopathological mechanisms under the activator effect of ramelteon on bladder smooth muscle. In addition, there are no studies examining the efficacy of ramelteon and used in lower urinary tract infections, on bladder contraction.

Conclusion

In conclusion, in our study, we examined the possible effects of ramelteon on bladder contractions and found that ramelteon exerts an activator effect on bladder contractions. It can be said that this effect may be effective in reversing bladder activity in patients with clinically atonic and areflexic bladder problems. Despite various pharmacological agent trials, especially for the medical treatment of atonic bladder, the development of a safe and selective agent for in vivo use has been difficult. In this study, it is thought that ramelteon is effective in inducing bladder contractions and may have beneficial effects in such patients. This effect should be taken into account in clinical use.

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