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Effect of agomelatine on intestinal smooth muscle contractility in rats

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Abstract

Aim: The purpose of the present study was to investigate in vitro the effect of agomelatine, an analog of melatonin, on potassium chloride-stimulated contractions (KCl) in duodenum, jejunum, ileum, and colon in rats.

Materials and Methods: A total of 7 Wistar albino female rat strips were used in the study. Rats were 8-10 weeks old and weighed 200-250 g. After the rats were decapitated without anesthesia, duodenum, jejunum, ileum, and colon strips were prepared and placed in the organ bath. Agomelatine was applied in doses of 100, 200, and 1000 µM to the strips that were contracted with KCl (80 mM). Then, 1000 µM agomelatine was applied after preliminary treatment of melatonin receptor antagonist luzindole (1 µM) to investigate the action mechanism of agomelatine on intestinal smooth muscles. The records were analyzed before and after each dose, and frequency, peak to peak (P-P) and area values were compared.

Results: Agomelatine inhibited the KCI- induced contractions in duodenum, jejunum, ileum, and colon strips in a dose-dependent manner. Depending on the agomelatine application, the frequency, "P-P", and the area values under the curve decreased at statistically significant levels ($p<0.05$). However, the effect of agomelatine decreased at significant levels as a result of luzindole treatment ($p<0.05$).

Conclusion: The dose-dependent inhibitor effect of agomelatine on KCl-stimulated contractions in the rat intestinal smooth muscle was shown for the first time in this study. Agomelatine was found to affect the smooth muscles of the intestines through melatonin receptors. This result suggests that agomelatine might have therapeutic potential in diseases in which gastrointestinal motility is impaired.

Introduction

Melatonin is a lipophilic hormone that is secreted in the dark from the pineal gland. Melatonin, which regulates circadian rhythms, is active in physiological processes, such as sleep-wake cycle, thermoregulation, pubertal development, seasonal adaptation, intestinal reflexes, and immunomodulation. It is also known that it has antinociceptive, anxiolytic, neuroprotective, antidepressant, locomotor activity regulator, blood pressure-decreasing, anti-inflammatory, tumor suppressor and antioxidant effects [1-4].

Melatonin was first isolated by Lerner from the pineal gland [5]. The most important extrapineal source of melatonin, which is also secreted in many non-pineal gland organs, are enterochromaffin cells in the gastrointestinal mucosa [1]. Melatonin is synthesized in these cells through serotonin from the tryptophan [1]. It is known that gastrointestinal melatonin is at least 400 times more than in the pineal gland [1, 6-8]. It is also 10-100 times more than the amount in circulation [1, 9, 10]. Unlike in the pineal gland, the change in the amount of gastrointestinal melatonin is not photoperiodic, but is related to nutrient intake [11, 12].

Melatonin exerts its effect through melatonin receptors (MT1, MT2 and MT3). Melatonin shows its effects

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through G-protein-clamped membrane receptors MT1, MT2 and MT3-bonding area, nuclear receptors, intracellular signaling proteins, and antioxidant mechanisms. Melatonin receptors were detected widely in various gastrointestinal tissues. Melatonin has effects on antioxidant reactions, secretion, and visceral sense in the gastrointestinal tract. It is also effective in regulating the gastrointestinal smooth muscle motility [1, 2, 4, 13, 14].

Agomelatine is a new melatonin analogue that is being used as an antidepressant [15]. It is a melatonergic agent that comes to the forefront for its length of half-life and higher affinity for MT1 and MT2 receptors compared to melatonin [16]. Agomelatine has agonist effects on MT1 and MT2 receptors, and antagonist effects on 5-HT2C receptors [15]. The dose-dependent effect of melatonin on intestinal motility was shown in the literature [18]. However, no studies were detected showing the in vitro effects and action mechanism of agomelatine on intestinal smooth muscle contractility. The purpose of this study was to show the effect and action mechanism of agomelatine on rat duodenum, jejunum, ileum and colon contractility.

Materials and Methods

The present study 7 Wistar albino female rat strips were used. Rats were 8-10 weeks old and weighed 200-250 g. which were taken from Firat University Experimental Research Center (FUDAM). This study was conducted at this unit in accordance with the guidelines for the ethical use of laboratory animals that were approved by the Firat University Ethics Committee of Experimental Animals Research as of October 23, 2019, 2019/200 (Elazig, Turkey). Rats were decapitated without anesthesia, and duodenum, jejunum, ileum and colon tissues were removed. Strips of 10 mm long and 3 mm wide were prepared from the tissues, and were placed in isolated tissue baths that contained Krebs Solution at 37°C gassed with 95% and 5% CO2. The tissues were balanced by washing with Krebs Solution every 15 min under 1 g of resting tension for an hour before the agomelatine administration. The feedback formed in the tissues was measured by using the MP150 transducers (Biopac Systems, Inc., USA). Contraction was stimulated with 80 mM KCl after an hour of stabilization, and 100 µM agomelatine was administered. After 10 minutes of recording, the tissues were washed 3 times with Krebs Solution. After repeating the 1-hour stabilization step, the experiment was repeated with a dose of 200 µM agomelatine, and 10 minutes of recording was carried out. After repeating the washing and stabilization steps, the experiment was repeated with a dose of 1000 µM agomelatine, and 10 minutes of recording. After repeating the washing and stabilization steps, 1000 µM agomelatine and luzindole (1 µM) was applied following pre-treatment. The process was then terminated after 10 minutes of recording. The records before and after each dose were analyzed. The frequency, "P-P", and AUC were compared.

Data analysis

Data are presented as means ± standard error of mean (SEM) for 7 animals. The number of animals to be used in the experiments; 8% deviation, type 1 error (α) 0.05 and type 2 error (β) (Power=0.80) and at least 7 animals were determined by power analysis. Relative changes of drug-induced (agomelatine and luzindole) contractile responses to the maximum levels (induced with KCl) were calculated as a percentage. Statistical analyses were performed using SPSS version 22 (IBM Corp, Inc., Authorization code: 794f5c72bc41572d732f , Chicago, IL, USA) and graphs were made using OriginPro 8 SR1 (OriginLab Co, Northampton, MA). For peak-to-peak amplitude and area values, the control group values were recognized as 100 percent, and the change was computed as percent (percent) for all treatment groups. Calculations for each dose were evaluated for periods of 10 min. The data were statistically analyzed using the one-way ANOVA test and paired sample t-test. All results with p value less than 0.05 in all analyzes were considered statistically significant.

Research ethics

The Firat University, Animal Experiments local Ethics Committee, permission number: 200, with protocol number: 2019/134 date: 23/10/2019, evaluated and approved this study procedure.

Results

In the present study, it was shown in vitro that agomelatine inhibits contractions stimulated by KCI in rat duodenum, jejunum, ileum and colon (Figure 1 and Figure 2). It was determined that this inhibition was dose-dependent in all tissues. The frequency, "P-P", and area values under the curve (AUC) decreased at statistically significant levels in all doses ($p<0.05$). This decrease was the most at the dose of 1000 µM agomelatine. The 200 µM agomelatine dose resulted in significant statistical decrease compared to 100 µM agomelatine ($p<0.05$). No significant differences were detected between 200 µM agomelatine and 1.000 µM agomelatine. Following the luzindole administration, the effect of agomelatine at a dose of 100 µM decreased significantly ($p<0.05$).

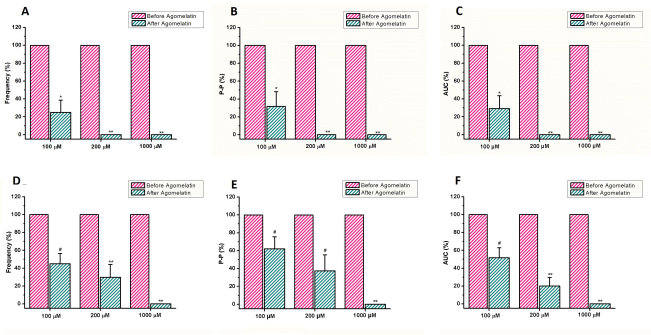

Figure 1. Effect of agomelatine on duodenum [A–C (Frequency=100 µM, p=0.036; 200 µM, p=0.002; 1000 µM, p=0.001; P-P= 100 µM, p=0.051; 200 µM, p=0.002; 1000 µM, p=0.001; AUC=100 µM, p=0.049; 200 µM, p=0.047; 1000 µM, p=0.024)] and jejunum [D–F (Frequency=100 µM, p=0.002; 200 µM, p=0.006; 1000 µM, p=0.001; P-P= 100 µM, p=0.021; 200 µM, p=0.021; 1000 µM, p=0.006; AUC=100 µM, p=0.007; 200 µM, p=0.043; 1000 µM, p=0.000)] contracted with KCl (#p<0.05, *p<0.01, **p<0.001).

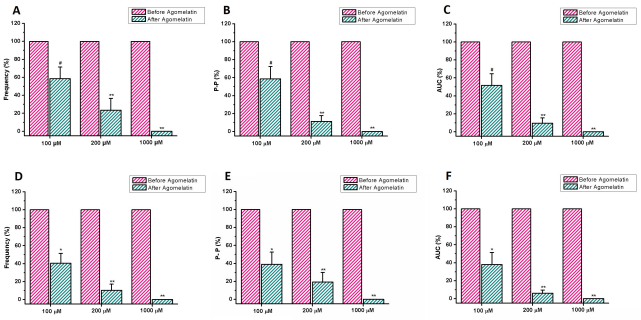

Figure 2. Effect of agomelatine on ileum [A–C (Frequency=100 µM, p=0.018; 200 µM, p=0.001; 1000 µM, p=0.000; P-P= 100 µM, p=0.143; 200 µM, p=0.033; 1000 µM, p=0.041; AUC=100 µM, p=0.117; 200 µM, p=0.037; 1000 µM, p=0.028)] and colon [D–F (Frequency=100 µM, p=0.001; 200 µM, p=0.013; 1000 µM, p=0.020; P-P= 100 µM, p=0.018; 200 µM, p=0.010; 1000 µM, p=0.017; AUC=100 µM, p=0.094; 200 µM, p=0.009; 1000 µM, p=0.021)] contracted with KCl (#p<0.05, *p<0.01, **p<0.001).

Discussion

In the present study, the effect of agomelatine, which is a new melatonin analogue, and which is used as an antidepressant, on intestinal smooth muscle contractility was investigated. It was shown for the first time that agomelatine suppresses the KCl-stimulated contractility in duodenum, jejunum, ileum and colon strips under in vitro conditions in a dose-dependent manner. It was also determined that agomelatine performs this effect through MT1 and MT2 receptors. The effects of melatonin on gastrointestinal smooth muscle were investigated in various studies. It was reported to have a contractile or relaxant effect in a dose-dependent manner.

Quastel and Rahamimof showed that melatonin inhibited spontaneous and serotonin-stimulated contractions in rat duodenum in a dose-dependent manner [19]. Harlow and Weekly reported that melatonin showed inhibitory activity on spontaneous contractility in the duodenum, jejunum, ileum and colon strips of rats [20]. Fioretti et al. determined that in vitro melatonin inhibited contractions in isolated rat stomach segments [21]. Bubenic showed that the application of in vitro melatonin in rats inhibited spontaneous or serotonin-stimulated ileum tonus, but did not cause changes in amplitude and frequency [22]. In this study, it was also observed that there were no changes in the intestinal contraction stimulated with Acetylcholine (Ach) after pre-treatment of melatonin [22]. Drago et al. found that low doses of intraperitoneal melatonin accelerated intestinal transition in rats, and slowed it at high doses [23]. It was also found in this study that intestinal myoelectrical activity increased with the application of intraperitoneal melatonin at low doses [23]. Kasimay et al. showed that intraperitoneal melatonin delayed gastric emptying in rats at pharmacological doses [24]. In this study, it was reported that this effect of melatonin was associated with CCK2 and 5HT3 receptors (receptor blockers) [24]. In the same study, it was also found that sympathetic neuron activation also played roles in this effect of exogenous melatonin on gastric motility [24].

Velarde et al. showed that melatonin relieved intestinal strips that was stimulated with serotonin and Ach; however, did not have effects on KCl-stimulated contractions in ornamental fish [25, 26]. They also reported that nitrergic pathway and extracellular calcium and melatonin receptors were effective in these effects [25, 26]. Barajas-Lopez et al. reported that melatonin specifically relieved gastrointestinal muscles by blocking nicotinic channels in guinea pigs [27]. Reyes-Vasquez et al. found that melatonin inhibited contractions stimulated by KCl or carbachol in a dose-dependent manner by interacting with K+ channels activated by apamin-sensitive Ca2+ (low conductance) in rat ileum strips [28]. As it is seen, there are studies in the literature using melatonin at low doses, as there are several other studies investigating the effects in contractions stimulated by agents, such as serotonin, KCl or Ach. Furthermore, some studies were conducted under in vivo conditions, and others used different species as subjects. The study presented here was conducted on rats by using organ bath method under in vitro conditions. In this study, the effect of agomelatine, which is an analogue of melatonin, on intestinal contractility was investigated for the first time. The commercial form of agomelatine was used by adapting the dose of treatment for humans to rats. It was shown for the first time that agomelatine inhibits stimulated contractions in the duodenum, jejunum, ileum and colon at pharmacological doses in a dose-dependent manner. Also, the mechanism of action was investigated, and it was revealed that MT1 and MT2 receptors mediated this effect of agomelatine.

The therapeutic potential of melatonin on some gastrointestinal pathologies where gastrointestinal motility is irregular was revealed in various clinical trials conducted on humans. Studies on Irritable Bowel Syndrome (IBS), which is a common pathology often reported to be accompanied by depression and anxiety [29] is particularly noteworthy. In these studies, Song et al. showed that abdominal pain decreased [30]; Saha et al. reported that IBS score showing quality of life improved [31]; Lu et al. argued that abdominal pain, distension, and abnormal defecation sensation improved [32]; and Chojnacki et al. said that abdominal pain and distension decreased [33]. Balakina et al., on the other hand, used agomelatine experimentally (its commercial form, valdoxan) for the treatment of irritable bowel syndrome (IBS), and found improvements in the symptoms of the disease, and an increase in quality of life [34]. These results show the importance of the regulatory effect of melatonin and agomelatine on gastrointestinal motility, and their therapeutic potential in exogenous use. The findings of the presented study suggest that agomelatine can be used in diseases related to gastrointestinal motility.

Conclusion

As a conclusion, melatonin analogues are becoming increasingly important in our present day, and their therapeutic potentials in many diseases is revealed. It is extremely important to investigate the effects of these agents on gastrointestinal motility and their mechanisms of action. In the study presented here, it was shown

that agomelatine, which is a new and powerful melatonin analogue, and which is used as an antidepressant, suppresses KCl-stimulated intestinal smooth muscle activity in duodenum, jejunum, ileum and colon regions in a dose-dependent manner. Also, the mechanism of action of agomelatine was investigated in this study, and it was revealed that it affects the intestinal smooth muscle activity through melatonin receptors. In our opinion, agomelatine may have beneficial effects in motility diseases, regulating the intestinal smooth muscle activity. We think that the findings of this study can shed light on new studies in this field.

Declaration of interest

The authors contributing to this research declare that there is no conflict of interest that could harm the objectivity of the research.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

SS, AY, NU, FT, OB and ZE performed the research, analyzed the data, wrote the original draft, and revised the manuscript; EK designed the research study, analyzed the data, wrote the original draft, and contributed essential reagents or tools, revised the manuscript, supervised and administered the project; IS analyzed the data, contributed essential reagents or tools. All authors approved the final version of the manuscript.

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Ethics approval

The Firat University, Animal Experiments local Ethics Committee, permission number: 200, with protocol number: 2019/134 date: 23/10/2019, evaluated and approved this study procedure.

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