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Role of cannabinoid receptor-2 in small intestinal fasted myoelectric activity of rats

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Abstract

Cannabinoid receptor-1 (CB_1R) and cannabinoid receptor-2 (CB_2R) have significant roles in the esophagus, small intestine, and colon motility in the postprandial period besides gastric emptying, secretion, and defecation in the gastrointestinal system. Furthermore, our previous study showed that activation of peripheral CB_1R inhibited migrating myoelectric complex (MMC), forming the source of fasted small intestinal motility. However, the role of the central/peripheral CB_2Rs on the MMC pattern is still unknown. The present study aimed to investigate the roles of peripheral and central CB_2Rs in forming and regulating small intestine MMC patterns in rats. In this study, we used 42 adult male Sprague-Dawley rats (n:7). We implanted bipolar electrodes in three different jejunum sites of rats (J1, J2, J3) to record the MMC pattern. We placed a cannula in the right lateral ventricle to perform drug intracerebroventricularly (i.c.v.) and implanted a catheter in the right jugular vein to inject the drug intravenously (i.v). After the amelioration period, we conducted experiments following an 18-hour fasting period and later took at least a one-hour baseline recording of the MMC pattern. Then, we injected JWH 133, a CB_2R agonist i.v. (1.25-10 mg/kg) or i.c.v. (2.5-20 µg/rat) and also administered AM 630, a CB_2R antagonist, i.v. (0.25-2 mg/kg) or i.c.v. (2.5-20 µg/rat). We compared the effects of JWH 133 or AM 630 on the MMC pattern to the vehicle group (10% dimethyl sulfoxide). Centrally or peripherally injected JWH 133 and AM 630 did not cause any change in the spike frequency and the number of the MMC cycle. The results of the present study propose that CB_2Rs are involved in neither endogenous formation nor exogenous regulation of the fasting myoelectric activity in healthy fasted rats.

Keywords: cannabinoids, cannabinoid receptor-2 (CB_2R), gastrointestinal motility, migrating myoelectric complex (MMC), rat

1. Introduction

Small intestinal motility mixes the chyme with digestive enzymes and bile secretion, and transports the content throughout the gut in coordination with the digestive and absorption functions. The postprandial pattern, which occurs in the small intestine following food intake, consists of segmentation and peristalsis. In the fasted state, a unique and complex pattern comes into play in the whole small intestine (1). This pattern consists of 3 phases in which the contractile activity at any point in the small intestine has shown recurrent differences. Firstly, there are not almost contractions for about 40-50 minutes (phase I), and later many irregular contractions occur for 30-40 minutes (phase II). Finally, intense contractions occur for 5-10 minutes (phase III). Then phase I begins again, and the cycle rhythmically continues approximately every 90-120 minutes until the next meal in human. The cycle starting in the most proximal part of the small intestine moves (migrates) towards the distal portion of the intestine (2). Because of its migration, this complex has been termed "the migrating motor/motility complex" by Foulk *et al.* in 1954 (3). The physiological significance of this complex is that it moves the undigested food residues, gastrointestinal (GI) secretions, and dead cells from the stomach and small intestines to the large intestine, having prevented the bacteria from transitioning from the large intestine to the small intestine. Because of these crucial functions, the migrating motor/motility complex is called the "housekeeping of the gastrointestinal tract" (4).

First, Szurszewski (1969) demonstrated that the electrical changes (slow and spike waves), which are recorded in the smooth muscle of the small intestine in dogs, forming the origin of the migrating motor/motility complex and also termed the "migrating myoelectric complex: the MMC" (5). Obviously, the control mechanism of the MMC is complex because it occurs rhythmically and regularly. Many neurohumoral factors play a role in the formation/ regulation of the MMC, such as mainly motilin, acetylcholine, somatostatin, pancreatic polypeptide, serotonin, xenin, and ghrelin (6-13). Furthermore, the enteric nervous system initiates, maintains, and coordinates the MMC while the parasympathetic/sympathetic nervous system is only involved

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in the coordination of the MMC (14-16).

The endocannabinoid system comprises endogenous ligands, synthesis/degradation enzymes, and primary receptors (cannabinoid receptor-1 and cannabinoid receptors-2). Endocannabinoid ligands, mainly anandamide and 2-AG, are produced on demand from membrane phospholipids in postsynaptic neurons. The ligands are released immediately without being stored in vesicles and then function as retrograde messengers (17, 18). Cannabinoid receptor-1 (CB_1R) is mainly expressed in the central nervous and enteric nervous systems, and one of its primary functions is modulating neurotransmitter release in several neurons (19). In contrast, cannabinoid receptor-2 (CB_2R) is mainly expressed in immune system organs, such as the thymus, and spleen and is often referred to as the peripheral cannabinoid receptor. The major receptors of endocannabinoids are seven-transmembrane G-protein-coupled receptors, leading to the suppression of adenylate cyclase activity (20, 21).

Endocannabinoid system components are present in the GI tract. Endogenous ligands or their pharmacological modulation (ligand, receptor agonists, inhibitor of catabolic enzyme) can cause inhibition in GI functions, such as gastric emptying, peristalsis, and defecation; conversely, suggesting that specific CB_1R/CB_2R blockage can lead to excitation of the functions (18, 22, 23). In the postprandial state, there is substantial evidence that CB_1R/CB_2R mediates the inhibitor effects of cannabinoids on GI motility. *In vitro* study results have shown that nonspecific CBR agonists and specific-CB_1R agonists activate CB_1R and reduce the amplitudes of smooth muscle contractions induced by electrical field stimulation in the gastric fundus, antrum, ileum, and colon preparations state (24-29). Similarly, *in vivo* studies supported that CB_1R activation has inhibited GI transit in the fed states (18, 30-32). Furthermore, particular CB_2R receptor agonists have been proven in multiple studies to reduce increased GI transit caused by various pathological conditions (e.g., inflammation, colitis, irritable bowel syndrome). Consistent with these findings, peripheral CB_1R activation has also inhibited the MMC pattern generating the origin of fasting motility (33).

However, it is unknown whether **(a)** central/peripheral CB_2Rs have a tonic role in the regulation/formation of the MMC and **(b)** whether central/peripheral CB_2R activation by exogenously administered CB_2R agonist affects the MMC pattern. For this purpose, to investigate the role of CB_2Rs in migrating myoelectric complex, we examined whether **(1)** central and peripheral administration of CB_2R antagonists and **(2)** central and peripheral administration of CB_2R agonists caused any change in the MMC pattern.

2. Material and Methods

2.1. Animals

We obtained adult male Sprague Dawley rats weighing 250 to 300 g from the Animal House of Ondokuz Mayıs University (Samsun, Turkey). We housed them in a quiet, temperature- and humidity-controlled room at 22±1°C for a 12-h alternating light-dark period, and gave food and water ad libitum. We conducted all protocols and procedures under the Guide for the Care and Use of Laboratory Animals (NIH Publication, 865–23, Bethesda, MD, USA).

2.2. Surgical procedures

We used ketamine [50 mg/kg; intraperitoneal (i.p)] and chlorpromazine (25 mg/kg; i.p) to anesthetize the male Sprague-Dawley rats and, through a midline incision, placed three bipolar stainless Ni/Cr wire electrodes (Driver-Harris, Cedex, France) into the muscular wall of the small intestine 15 (J1), 25 (J2), and 35 (J3) cm distal to the pylorus for electromyographic (EMG) recordings. We placed a catheter in the right jugular vein to reveal the peripheral effect of the substance, and a 24-gauge cannula in the right lateral ventricle (1.5 mm caudal, 2 mm lateral from Bregma; 6 mm ventral from the skull surface) to administer drug centrally. We tunneled the electrodes and cannula subcutaneously to exit the back of the animal's neck. Later, we tunneled both the EMG electrodes and the catheter subcutaneously and externalized them at the back of the animal's neck. We then fixed the EMG electrodes to the skull with dental acrylic. We housed all the male rats, one in each cage after surgery. We treated the rats with antibiotics (Ampicillin; 100 mg/kg; intramuscular) and analgesics (Metamizole Sodium; 100 mg/kg; i.p.) for the first three days following surgery (34).

We adapted the rats to the experimental conditions by putting them in Bollman cages (Bahadır Co., Turkey). We performed this putting process for 2 hours a day until the experiment day (7th post-operative day).We made the rats hungry for 18 hours without water restriction in wire-bottomed cages before experiments for EMG recording (12, 14, 33). We performed all the experiments on conscious rats in the Bollman cages. We recorded and analyzed EMG recordings amplified with a bioamplifier (ML132, ADInstruments, Australia) by the PowerLab data acquisition system (ML870/P, PowerLab 4/SP, AD Instruments, Castle Hill, NSW, Australia)

2.3. Design of electromyography studies

The experiments started with a control recording of baseline myoelectric activity with three MMC cycles propagated over all three sites (J1, J2, J3) for at least one hour. We started administering the drug at the end of the fourth MMC cycle at J1 sites. After drug application, we took recordings for a minimum of one hour. We tested each animal was tested 2-5 times with an interval of 3 days.

- In the first series of experiments, we injected a vehicle (10% DMSO) intravenously (i.v.) in a volume of 1 ml/kg (n=7).
- In the second series of experiments, we injected a vehicle (10% DMSO) intracerebroventricularly (i.c.v.) in a volume of 5 µl/rat (n=7).

- In the third series of experiments, we injected JWH 133, CB_2R agonist, at doses of 1.25-10 mg/kg intravenously (n=7).
- In the fourth series of experiments, we injected JWH 133, CB_2R agonist, at doses of 2.5-20 µg/rat intracerebroventricularly (n=7).
- In the fifth series of experiments, we administered AM 630, CB_2R antagonist, at doses of 0.25-2 mg/kg intravenously (n=7).
- In the sixth series experiment, we administered AM 630, CB_2R antagonist, at doses of 2.5-20 µg/rat intracerebroventricularly (n=7).

2.4. Drugs and chemicals

We prepared all drugs on the experimental days just before drug administration. We dissolved agonists and antagonists were in 10% dimethyl sulfoxide (Sigma, St. Louis, MO). We injected i.c.v. with a Hamilton syringe in a volume of 5 µl/rat, and administered i.v. within a volume of 1 ml/kg with an insulin syringe. We purchased from JWH 133 (CB_2R-selective agonist) and AM 630 (CB_2R-selective antagonist) from PolyPeptide (Strasbourg, France). We selected the agonist and antagonist doses investigated in our study by considering the doses of these agents that are effective on the GI system in the literature (35-41).

2.5. Data analysis

We characterized the typical feature of intestinal myoelectric activity in the interdigestive period, or phase III of the MMC, as a period of clearly distinguishable intense spike waves, propagating aborally through the all recording segment and followed by a period of quiescence, phase I of the MMC having only slow waves and identified phase II of the MMC as a period of irregular spike waves. Within 1 hour after drug administration, we calculated spike frequency and numbers of the MMC cycles in all three sites (J1, J2, and J3) and analyzed them with the LabChart 7.0 program (12, 33).

2.6. Statistics

We converted all of the obtained EMG recordings to numerical values and used GraphPad Instat (v3.06) software for statistical analysis (San Diego, CA, USA). After determining that all the data were normally distributed, we performed a one-way analysis of variance (ANOVA) and Tukey–Kramer post hoc tests for multiple comparisons between groups. We expressed all values used in the graphics as mean±standard error (SEM). We considered $p< 0.05$ was considered statistically significant for all statistical tests,

3. Results

In the interdigestive period, all animals showed a fasted motor motility with recurrent MMC cycles propagated to the distal intestinal segments. Fig. 1 shows the characteristics of the MMC pattern at all theree jejunum sites in the EMG recording.

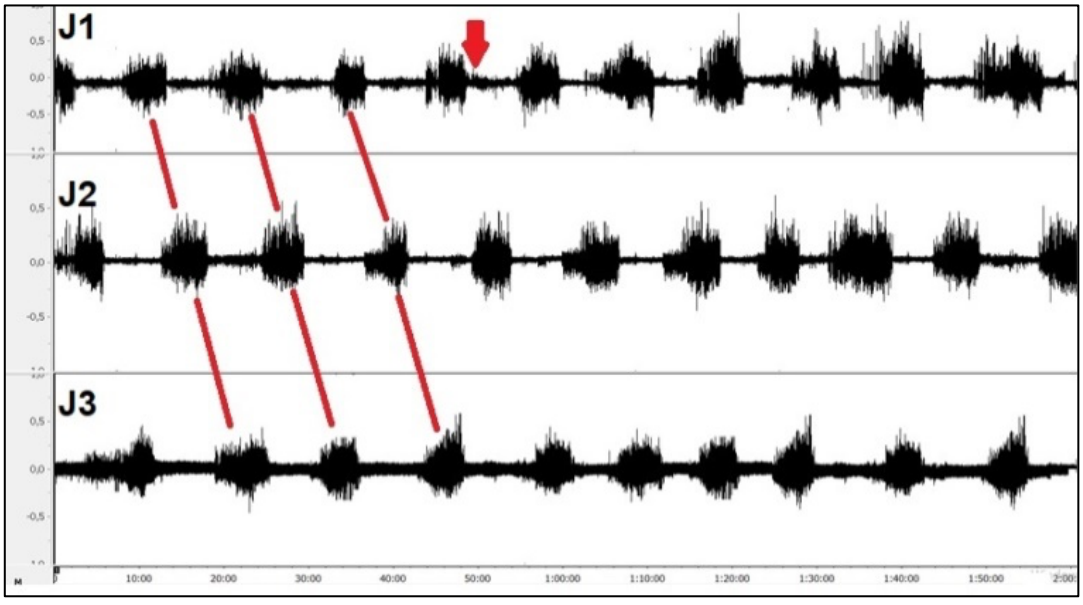

Fig. 1. In a conscious and fasted rat, three regular the MMC cycles at all three points (red lines) and drug injection at the end of the 4th the MMC cycle at J1 point (red arrow)

3.1. Effects of exogenously administered JWH 133, *CB_2R agonist,* on fasting myoelectric activity by activation of peripheral/central CB_2Rs

The effect of intravenously administered JWH 133 on the MMC pattern

Intravenous 10% DMSO administration (1 ml/kg), the solvent of JWH 133, did not change spike frequency and numbers of the MMC cycles at J1, J2, and J3 sites within one hour after administration compared to the baseline recording period. We, therefore, compared the effects of JWH 133 doses (1.25-10 mg/kg, i.v.) on the MMC pattern to the i.v. vehicle group. After i.v. injection of the CB_2R selective agonist JWH 133 at

1.25-10 mg/kg, it did not cause a statistically significant change in spike frequency at all three jejunal sites (Fig.2a). Additionally, the number of the MMC cycles remained unchanged by i.v. administered JWH 133 at jejunal recording sites (Fig. 2b).

The effect of intracerebroventricularly administered JWH 133 on the MMC pattern

After 10% DMSO injection (5 µl/rat, i.c.v.), the MMC pattern on baseline recording remained unchanged at jejunal recording sites compared to the control period. For this reason, we compared i.c.v. administrations of JWH 133 doses (2.5-20 µg/rat) on the MMC pattern according to the vehicle group. JWH 133 administered intracerebroventricularly, at doses of 2.5-20 µg/rat, did not cause a statistically significant change in spike frequency and the number of the MMC cycles in all recording sites compared to the vehicle group (Fig. 3a and b).

3.2. Tonic involvement of CB_2Rs in formation/regulation of fasting myoelectric activity

The effect of intravenously administered CB_2R antagonist AM 630 on the MMC pattern

After intravenous administration of AM 630, at doses of 0.25-2 mg/kg, it did not induce a statistically significant alteration in spike frequency at all three jejunal sites (Fig. 4a).

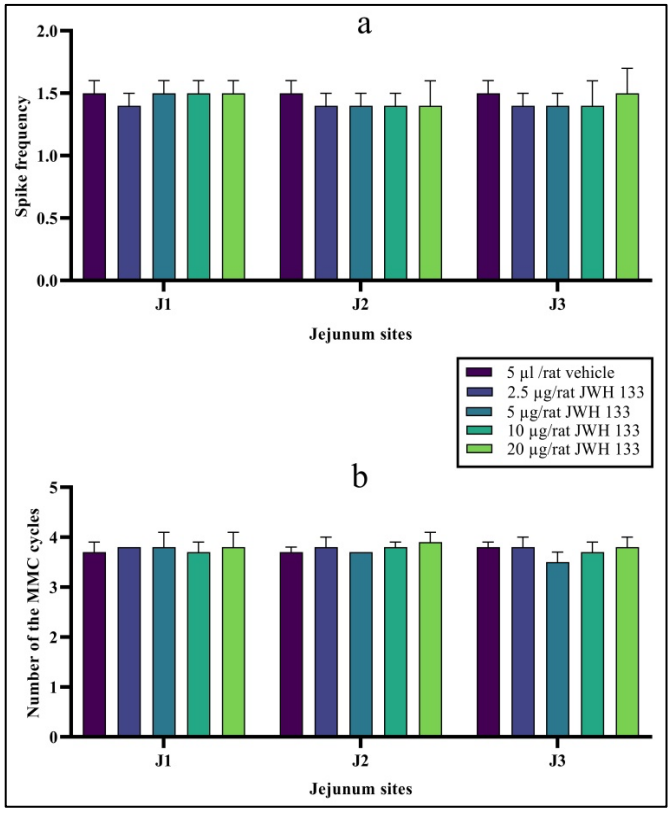

Fig. 3. Comparison of effect of different intracerebroventricular doses of CB_2R selective agonist, JWH 133, on the MMC pattern in its spike frequency **(a)** and number of the MMC cycle **(b)** (Mean±S.E.M., n=7)

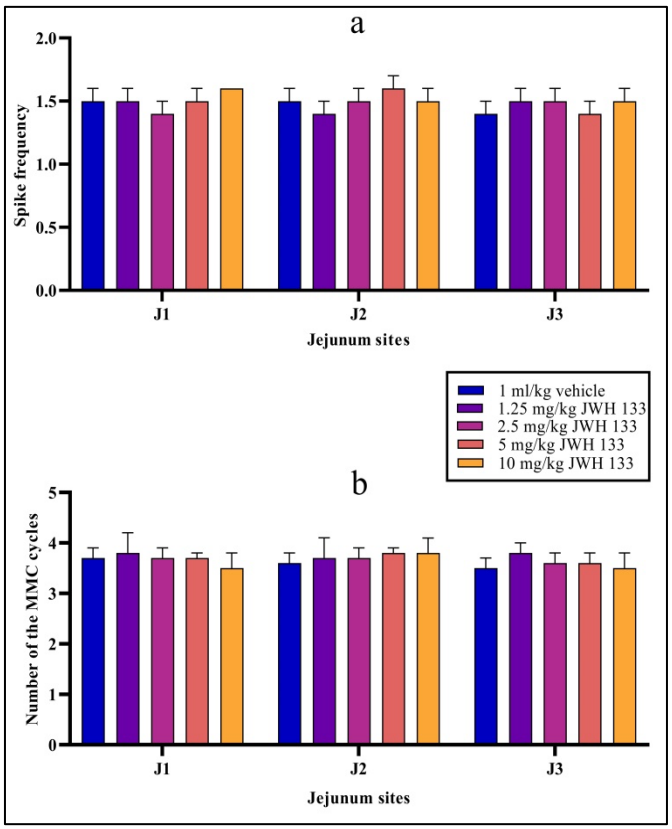

Fig. 2. Comparison of effect of different intravenous doses of CB_2R selective agonist, JWH 133, on the MMC pattern its spike frequency **(a)** and number of the MMC cycle **(b)** (Mean±S.E.M., n=7)

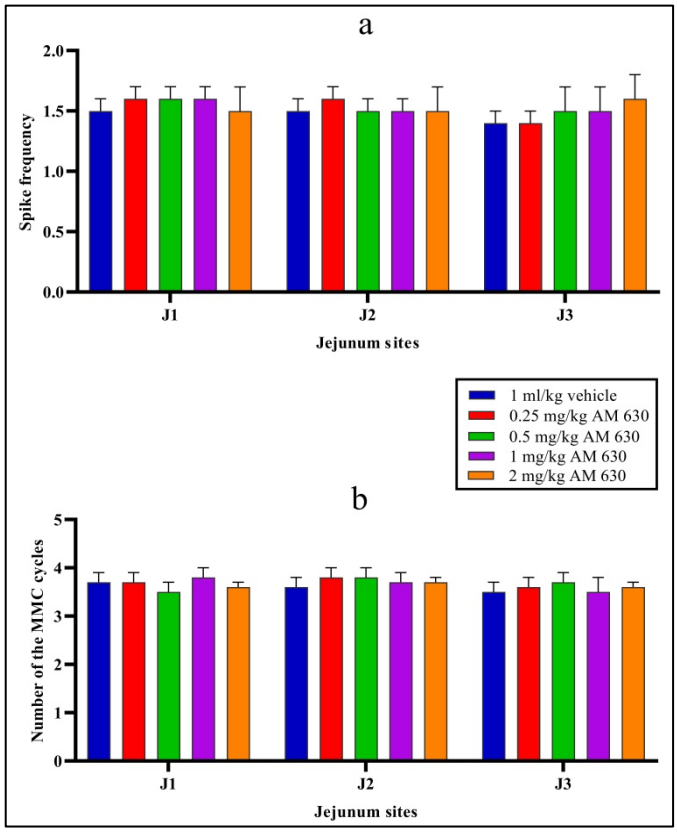

Fig. 4. Comparison of effect of different intravenous doses of CB_2R selective antagonist, AM 630, on the MMC pattern in its spike frequency (a) and number of the MMC cycle **(b)** (Mean+S.E.M., n=7)

Also, the number of the MMC cycles at J1, J2, and J3 sites did not alter by i.v. administration of AM 630 dosages compared to the vehicle group (Fig. 4b).

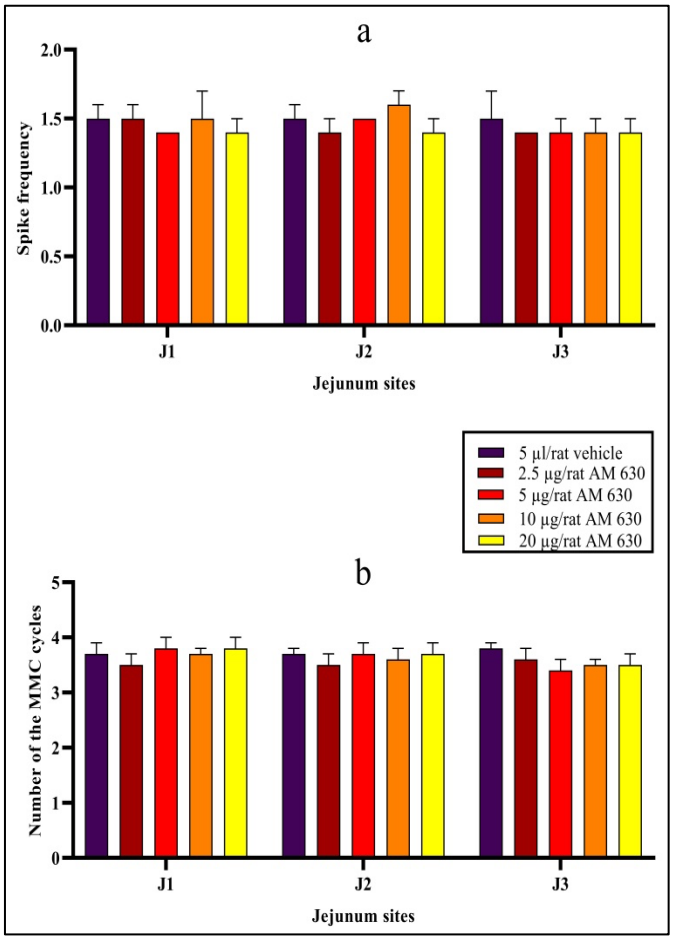

Fig. 5. Comparison of effect of different intracerebroventricular doses of CB_2R selective antagonist, AM 630, on the MMC pattern in its spike frequency **(a)** and number of the MMC cycle **(b)** (Mean+S.E.M., n=7)

The effect of intracerebroventricularly administered CB_2R antagonist AM 630 on the MMC pattern

Following approximately a one-hour baseline recording of i.v. AM 630 injection (2.5-10 µg/rat), neither the spike frequency nor the number of the MMC cycles were affected at J1, J2, and J3 sites compared to the vehicle group (Fig. 5a and b).

4. Discussion

Our study investigated the roles of CB_2Rs, which function in the gastrointestinal tract, in forming and regulating of the MMC in rats. For this purpose, we investigated the possible effect of CB_2R agonists and antagonists on the MMC pattern. Intravenously and intracerebroventricularly administrations of CB_2R agonist JWH 133 and CB_2R antagonist AM 630 were ineffective on the MMC pattern, the spike frequency and the number of the MMC cycles.

Many studies have shown the presence of endocannabinoid system components in the gastrointestinal tract. Mechoulam *et al.* (1995) isolated 2-AG in the canine intestine (42). Izzo *et al.* (2001) demonstrated the presence of anandamide in the mouse's small intestine (43). Moreover, the enzyme FAAH, responsible for breaking down anandamide, was observed in the intestines of mice and rats (44-46). CB_1R expression has been demonstrated in the enteric nervous system and epithelial cells by immunohistochemical studies, revealing that these receptors are associated with intrinsic primary afferent neurons, motor neurons, and interneurons of the GI tract (46, 47).

On the other hand, CB_2Rs are located in macrophages, plasma cells, and epithelial cells in the GI tract, suggesting that their expression increases in the inflammation process, indicating CB_2Rs function in the GI system in pathological conditions rather than physiological states (48-50).

According to the results of *in vitro* studies, endocannabinoids, phytocannabinoids, or synthetic cannabinoids bind to CB_1Rs on enteric neurons, leading to a decrease in the release of acetylcholine from neurons and, finally, inhibition of contractile responses (51-56). Similarly, *in vivo* animal studies shown that it suppresses small intestine and colon transit and defecation emerging in the fed period through the activation of CB_1Rs (56-59). Likewise, in our previous study, the CB_1R-specific agonist ACEA dose-dependently caused an inhibitory effect on the MMC pattern via the activation of peripheral CB_1Rs. Activation of peripheral CB1Rs not only exerts an inhibitory effect on postprandial motility but also has an inhibitory effect on fasting myoelectrical activity (33).

Many animal and human studies suggest that CB_1Rs act as inhibitor receptors on postprandial motility, but there is no involvement of CB_2Rs (18, 31, 32, 37, 59). However, it was unknown whether CB_2Rs participate in fasting intestinal motility activity. Therefore, we investigated here the role of CB_2Rs on the MMC. CB_2R agonist JWH 133 administered peripherally (1.25-10 mg/kg, i.v.) and centrally (2.5-20 µg/rat, i.c.v.) did not cause any change in the MMC in the present study in coherence with the studies in the literature. Furthermore, we administered CB_2R antagonist AM 630 both peripherally (0.25-2 mg/kg, i.v.) and centrally (2.5-20 µg/rat, i.c.v.); however, the antagonist did not affect the MMC pattern. Therefore, when evaluated together with the experimental results mentioned above, these findings suggest that CB_2Rs are not involved in both formation and regulation of the MMC as well as postprandial intestinal motility.

Although it was believed that CB_2Rs do not have a function in intestinal motility, the data revealed in recent years suggest that CB_2Rs have a role in intestinal motility changing in pathophysiological conditions rather than physiological states. Mathison *et al*. (2004) reported the CB_2R agonist JWH 133 decreased liposaccharide-induced intestinal transit in rats (39). Kimball *et al*. showed that JWH 133 normalized the accelerated transit in a colitis model in mice (40, 41). Lastly, Lin *et al.* (2019) observed that administering a new specific CB_2R agonist, AM 1241, returns to basal colon

motility in rats with diarrheal-irritable bowel syndrome (60). For this reason, it can considered normal that CB_2Rs did not participate in basal MMC activity in healthy fasted rats in this study.

In conclusion, the results of the present study suggest that CB_2Rs do not have tonic participation in the formation or regulation of MMC. Moreover, exogenous CB_2Rs activation does not have a role in the regulating of MMC forming source of fasting motility activity in healthy fasted rats.

Conflict of interest
None to declare.

Acknowledgments
None to declare.

Ethical Approval
The study was approved by the Ethics Committee of Ondokuz Mayıs University (date: 05.06.2020, No.2020/22).

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