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Effects of Cannabinoids on Colonic Muscle Contractility and Tension in Guinea Pigs

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Abstract

Objective: While endogenous cannabinoids regulate various physiologic functions, their role in the intestinal tract is unclear. We continuously recorded colonic motility in conscious guinea pigs. Mechanisms of action then were investigated using guinea pig taenia caecum in vitro.

Design: Prospective experimental observations using the cannabinoid agonists 2-arachidonoylglycerol (2-AG) and WIN55212-2; a cannabinoid antagonist, AM281; and ion-channel antagonist.

Setting: University research laboratory

Subjects: Thirty guinea pigs (20 for in vivo study, 10 for in vitro)

Measurements and main results: Colonic motility was monitored in vivo using telemetry via a force transducer attached to the guinea pig taenia caecum. Taenias isolated from other guinea pigs were studied in vitro to assess cannabinoid effects on muscle contractions evoked pharmacologically or electrically. Immediately after cannabinoid injection in conscious guinea pigs, taenial relaxation began peaking at 30 to 40 min. In animals pretreated with AM281, a CB1 cannabinoid receptor antagonist, cannabinoid evoked relaxation was less evident. In vitro, cannabinoids suppressed KCl-induced taenial contractions; this suppression was opposed by charybdotoxin, a Ca^{2+}-activated K^{+}-channel inhibitor, but not AM281. Cannabinoids decreased amplitude of repeated contractions evoked by electrical stimulation (an effect inhibited by AM281) but not muscle tension.

Conclusions: Cannabinoids decreased intestinal tract tension in vivo, apparently via central CB1 receptors. This differs from peristaltic suppression.

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Key words: cannabinoids, guinea pig, colon, contractility, tension, CB1 recepter

Introduction

As sepsis is particularly likely to develop in patients who require intensive care, these patients must be monitored closely for its occurrence. Severe sepsis is reported to carry a 40% risk of mortality[1]. Gastrointestinal stasis, which occurs frequently in patients with sepsis or multiple organ failure, has been linked to various complications and increased

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mortality. Such stasis induces bacterial overgrowth and impairs intestinal mucosal function. Consequently, stasis is thought to contribute to bacterial translocation[2], which leads to septic conditions that can culminate in sepsis lenta or recurrent sepsis. Increased plasma cannabinoid concentrations, presumably of endogenous origin, recently have been reported in patients with sepsis; cannabinoids therefore have drawn attention as an early mediator in septic shock[3]. However, an association between cannabinoids and gastrointestinal stasis has not yet been elucidated.

Effects of cannabinoids on the intestinal tract have been examined in vitro in terms of contractions of guinea pig ileal longitudinal and circular muscles evoked by electrical stimulation. This has shown that prejunctionally located cannabinoid CB1 receptors in smooth muscle inhibit release of acethylcholine (ACh) from intestinal nerves[4,5]. Whether cannabinoid receptors regulate bowel motility under physiologic conditions is unclear, although in vivo study reported that endogenous cannabinoids slowed colonic propulsion in mice[6]. To our knowledge, motility of intestinal smooth muscle has not been directly measured with respect to cannabinoid effects.

In this study we examined changes in colonic tension in response to cannabinoid agonists, WIN55212-2 and 2-arachidonoylglycerol (2-AG), in guinea pigs and contractile response in isolated colonic muscle, as indications of possible endogenous cannabinoid involvement in colonic motor regulation.

Materials and Methods

1) In Vivo Recording of Longitudinal Muscle Movement in the Caecum

Male Hartley guinea pigs (weight, 350 to 400 g) were used for experiments after approval of the Animal Experimental Ethical Review Committee of Nippon Medical School (No. 15～17). Guinea pigs were chosen for our in vivo experiment, because their sensitivity to endotoxin is comparable to that in humans[7]. Guinea pigs underwent laparotomy under anesthesia with pentobarbital sodium (30 mg/kg, i.p.). Longitudinal muscle movement was recorded using a method previously described by Ninomiya et al[8]. Briefly, a force transducer (3×5 mm, F-041S, Star Medical, Tokyo, Japan) was sutured to the taenia caecum. A cylindical electric transmitter (15×35 mm, IMT-10T; Star Medical) connected to the transducer via a cable was embedded subcutaneously in the dorsal region of the animal and sutured in place.

Signals from the transmitter were detected by a receiver (IMT-10RA; Star Medical) directly under the animal cages. Received output was stored in a personal computer, providing a continuous record of changes in tension of the longitudinal muscle. After surgical application of a force transducer to the caecum under anesthesia, the guinea pigs became conscious and physically active in their cages and consumed water and food. After becoming conscious, the animals showed a gradual increase in contractility and tension of the colonic longitudinal muscle, which reached a stable levels in the following several hours. Intestinal motility then was maintained, showing a regular pattern and minimal diurnal variation. Cannabinoid-agonist, WIN55212-2 (1 mg/kg) or 2-AG (1 mg/kg), was injected intraperitoneally at indicated doses 4 to 5 days after the operation, as longitudinal muscle movement of the taenia was recorded continuously. These doses have been shown to regulate colonic propulsion in mice[6]. Three or four animals were used for each dose of cannabinoid.

2) Measurement of Body Temperature

After depilation between the right and left scapulae on the dorsum of the animal, a plate-type thermosensor (PTP-50; Unique Medical, Tokyo, Japan) was fixed tightly to the skin. Skin temperature was recorded continuously by a temperature monitor (PTC-301; Unique Medical).

3) In Vitro Preparation

Male Hartley guinea pigs weighing 350 to 450 g were anesthetized with pentobarbital sodium (40 mg/kg, i.p.) and exsanguinated. Segments of the excised taenia caecum, about 1 cm in length, were mounted vertically in organ baths containing 5 m*l* of Krebs-HEPES solution at 32℃ and oxygenated with

100% O_2. The composition of the Krebs-HEPES solution (as mM) was: NaCl, 110.5; KCl, 5; $CaCl_2$, 1.2; NaH_2PO_4, 1: Glucose, 11.5; and HEPES, 24.5, at pH 7.4.

4) Evoked Isotonic Contractions

Mechanical activity of the isolated longitudinal muscle was recorded with an isotonic transducer (TD-112S; Nihon Koden, Tokyo, Japan) under a loading tension of 1 g. In some experiments contractions were induced ACh (10^{-5}M) or KCl (20 mM). These doses have been shown to produce sub-maximal contractile responses in our preliminary experiments (data not shown in present paper). In other experiments, contractions were evoked by electrical field stimulation (single bipolar rectangular pulses of 110% maximal voltage, 0.5 ms duration, 10 Hz frequency, 10 s train duration 2 min train frequency) applied through two platinum wire electrodes attached to the lower end of the longitudinal muscle segment and the upper end of the organ bath. Stimuli were generated by an electronic stimulator (SEN-7203; Nihon Koden, Tokyo, Japan) and resulting contractions were registered on a polygraph recorder (U-228; Nihon Koden) with an isotonic transducer (TD-112S; Nihon Koden). After stabilization about 2 h later, cannabinoids were added cumulatively at intervals of 20 min. Antagonists were applied 10 min before application of agonists.

5) Drugs

The following drugs were used: acetylcholine hydrochloride (Daiichi Seiyaku, Tokyo, Japan); 2-AG (2-arachidonoylglycerol, Cayman Chemical, Ann Arbor, MI); AM281 [(1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-4-morpphonyl-1H-pyrazole-3-carboximide, Tocris Cookson, Bristol, UK); AM630 {6-indo-2methyl-1-[2-(4-morpholinyl) ethyl]-1H-indo-3-yl (4-methoxyphenyl) methanone, Tocris Cookson}; charybdotoxin (Peptide Institute, Osaka, Japan), HEPES [(N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid) potassium hydrochloride, Wako Pure Chemical Industries, Osaka, Japan], pentobarbital sodium (Schering Plough, Kenilworth, NJ); WIN55212-2 mesylate {(R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo [1,2,3-de]-1,4 benzoxazin-6-yl] ± 1-naphathalenylmethanone mesylate, Tocris Cookson}.

The 2-AG originally was received in acetonitrile, which was evaporated under a gentle stream of nitrogen. For in vitro administration, 2-AG, WIN55212-2, AM281 and AM630 were dissolved in 100% dimethyl sulfoxide (DMSO). The contraction of the solvent in experiments never exceeded 0.1% (v/v). This final concentration of solvent had no effect on the muscle contractility. Other drugs were dissolved in distilled water. For in vivo experiments, 2-AG, WIN55212-2, AM281, and AM630 were dissolved in a mixture of ethanol, Tween 80, and saline (1 : 1 : 18) immediately before intraperitoneal administration.

6) Statistical Analysis

All experimental data are shown as the mean ± SEM. Statistical significance of differences was determined by repeated measure analysis of variance (ANOVA). Paired *t*-tests were used to evaluate differences between two groups. P-values less than 0.05 were considered indicative of statistical significance.

Results

1. Effects of WIN55212-2 and 2-AG on Motility of Colonic Longitudinal Muscle in Conscious, Unconstrained Guinea Pigs

In guinea pigs whose intestinal tension had remained stable for 4 to 5 days postoperatively, intraperitoneal administration of 2-AG (1 mg/kg) or WIN55212-2 (1 mg/kg) immediately induced a marked decrease in tension of the colonic longitudinal muscle (**Fig. 1**), but not its vehicle (data not shown).

The 2-AG-induced decrease in tension of the colonic longitudinal muscle was concentration-dependent and transient (**Fig. 2A**). In animals treated intraperitoneally with AM281 (3 mg/kg; a CB1 receptor antagonist) 10 min before administration of 2-AG (1 mg/kg), 2-AG-induced relaxation of the colonic longitudinal muscle was almost completely suppressed (**Fig. 3A**). AM630, a CB2 receptor antagonist did not suppress relaxation.

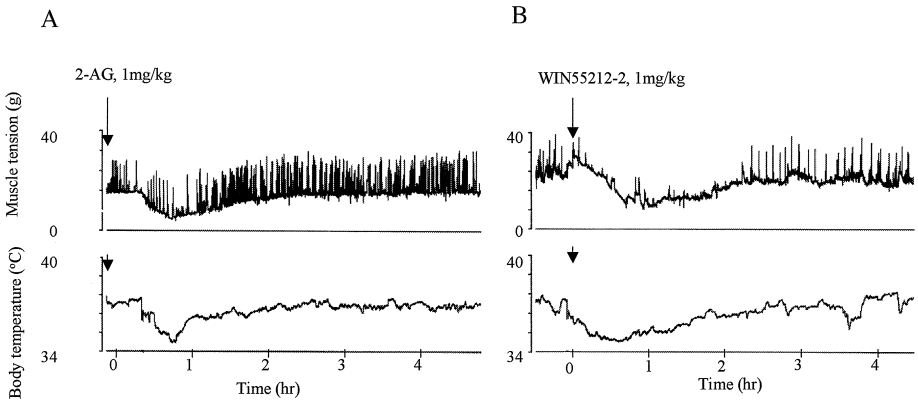

Fig. 1 Typical recordings of muscle tension and body temperature induced by injection of cannabinoids. Individual guinea pigs were injected intraperitoneally with 1 mg/kg of 2-AG (A) or 1 mg/kg of WIN55212-2 (B) at the time points indicated by arrows. Upper trace shows muscle tension (g) of the taenia caecum. The lower trace shows body temperature (℃). The horizontal axis shows time (hr) after administration of cannabinoids.

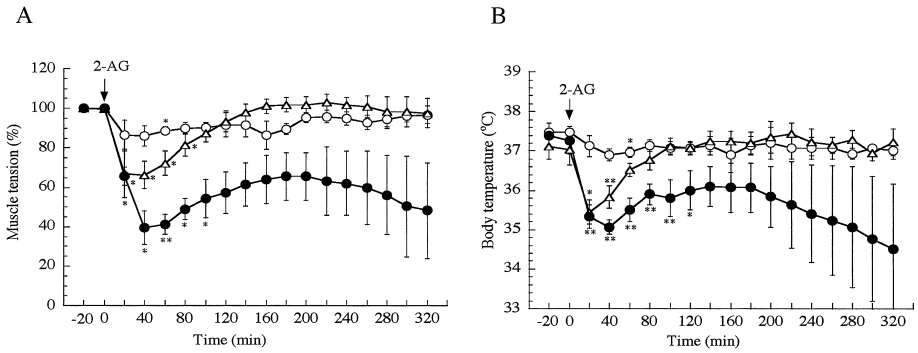

Fig. 2 Effects of 2-AG on muscle tension (A) of taenia caecum and body temperature (B) in guinea pigs. Individual guinea pigs were injected with 2-AG at 0.3 mg/kg (open circles), 1 mg/kg (open triangles), or 3 mg/kg (filled circles) intraperitoneally at time point 0. *$p<0.05$, **$p<0.01$ compared with the corresponding point at 20 min before injection of 2-AG. Points and bars respectively show the mean ± SEM (n = 3～4 per group).

(data not shown).

2. Effects of WIN55212-2 and 2-AG on Body Temperature in Conscious, Unconstrained Guinea Pigs

Body temperatures were monitored concomitantly with colonic longitudinal muscle tension. Immediately after receiving 2-AG (1 mg/kg) or WIN55212-2 (1 mg/kg), these animals showed a decrease in body temperature accompanying the decrease in colonic longitudinal muscle tension (**Fig. 1**). The 2-AG-induced decrease in body temperature was concentration-dependent and transient (**Fig. 2B**).

In animals treated intraperitoneally with AM281 (3 mg/kg), a CB1 receptor antagonist, 10 min before administration of 2-AG (1 mg/kg), 2-AG-induced hypothermia was suppressed (**Fig. 3B**). No such effect was observed with AM630, a CB2 receptor antagonist (data not shown).

3. Effects of WIN55212-2 on Muscle Contractions Induced by ACh or KCl in Vitro

Guinea pig taenia caecum was suspended in an organ bath with additions of WIN55212-2 (10^{-10} to 10^{-6} M) or 2-AG (10^{-10} to 10^{-6} M). This treatment did

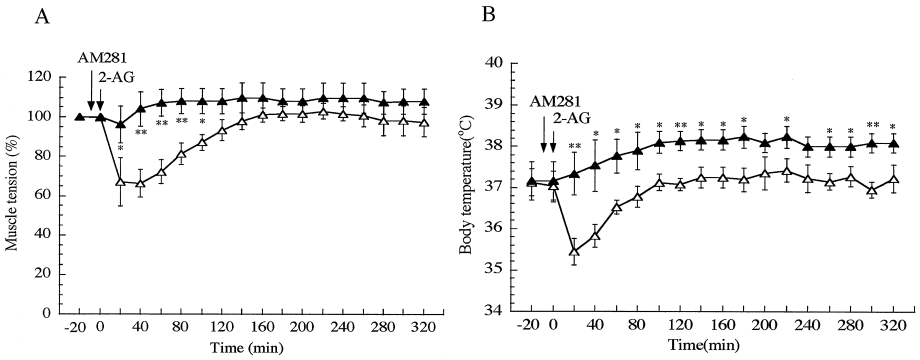

Fig. 3 Effects of AM281 on the muscle relaxation (A) and hypothermia (B) induced by 2-AG. Individual guinea pigs were injected with 2-AG at 1 mg/kg (open triangles) intraperitoneally at time point 0. AM281 3 mg/kg (filled triangles) was injected intraperitoneally 10 min before injection of 2-AG. $^{*}p<0.05$, $^{**}p<0.01$ compared with the corresponding point 20 min before injection of 2-AG. Points and bars respectively show the mean ± SEM (n = 3～4 per group).

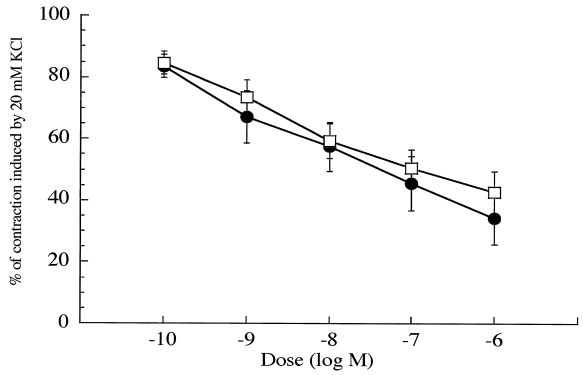

Fig. 4 Mean concentration-response curves for 2-AG (open squares) or WIN55212-2 (filled circles) on contraction induced by KCl at 20 mM in the guinea pig taenia caecum. The ordinate shows the percentage of contraction induced by 20 mM KCl. Points and bars respectively show the mean ± SEM (n = 4～7 different muscle preparations).

not affect mechanical activity of the smooth muscle.

The muscle preparation was treated with ACh (10^{-5} M) for induction of contractions. The ACh-induced contractions remained unaffected despite cumulatively increasing exposure to WIN55212-2 (10^{-10} to 10^{-6} M) or 2-AG (10^{-10} to 10^{-6} M), beginning 10 min after treatment with ACh.

The muscle preparation also was treated with KCl (20 mM) for induction of contractions. After the muscle was allowed to stand for 10 min until achieving stable tonus, it was exposed to 2-AG (10^{-10} to 10^{-6} M) or WIN55212-2 (10^{-10} to 10^{-6} M). Both compounds caused relaxation in a concentration-dependent manner (**Fig. 4**); their ED_{50} values for relaxation were 1.12×10^{-7} and 3.98×10^{-8} M, respectively. This induction of relaxation was not inhibited by AM281 (10^{-6} M), or AM630 (10^{-6} M), but was inhibited by charybdotoxin (10^{-8}M), an inhibitor at Ca^{2+}-activated K^{+}channel (**Fig. 5**).

4. Effects of WIN55212-2 on Muscle Contractions Evoked by Electrical Stimulation

When guinea pig taenia caecum suspended in an organ bath was electrically stimulated, single contraction was observed after single stimulus and repeated contractions of consistent amplitude were observed after each stuimulus given at 2 min intervals. The amplitude of these contractions was markedly decreased in the presence of atropine (10^{-7} M), and decreased to a negligible level in the presence of tetrodotoxin (10^{-6} M) although peristalsis was still observed. Papaverine (10^{-4} M) abolished both peristalsis and tonus (**Fig. 6**). Amplitudes of these contractions evoked by electrical stimulation were decreased by WIN55212-2 (10^{-8} to 10^{-6} M) in a concentration-dependent manner (**Figs. 6 and 7**). The WIN55212-2-related decrease in amplitude of contractions was prevented by AM281 (10^{-6} M; **Fig. 7**), but not by AM630 (10^{-6} M).

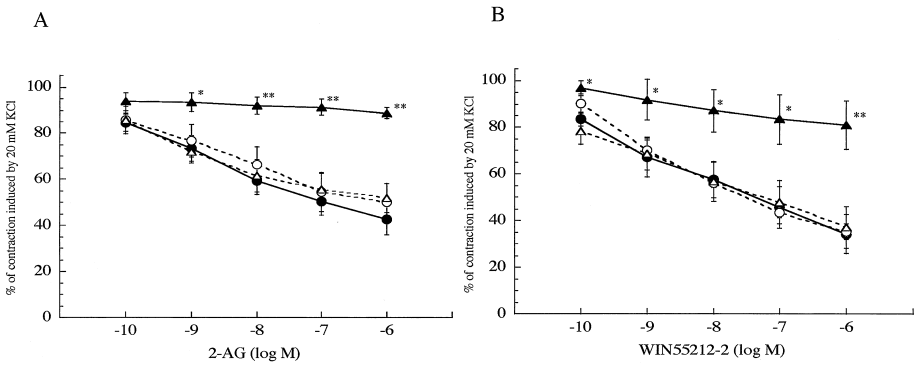

Fig. 5 Inhibitory effect of 2-AG (A) or WIN55212-2 (B) on contraction induced by KCl in guinea pig taenia caecum either alone (control, filled circles) or in the presence of AM281 10^{-6}M (open circles), AM630 10^{-6}M (open triangles), or charybdotoxin 10^{-8}M (filled triangles). *$p<0.05$, **$p<0.01$ compared with the corresponding point in control. Points and bars respectively show the mean±SEM (n=3～7 different muscle preparations).

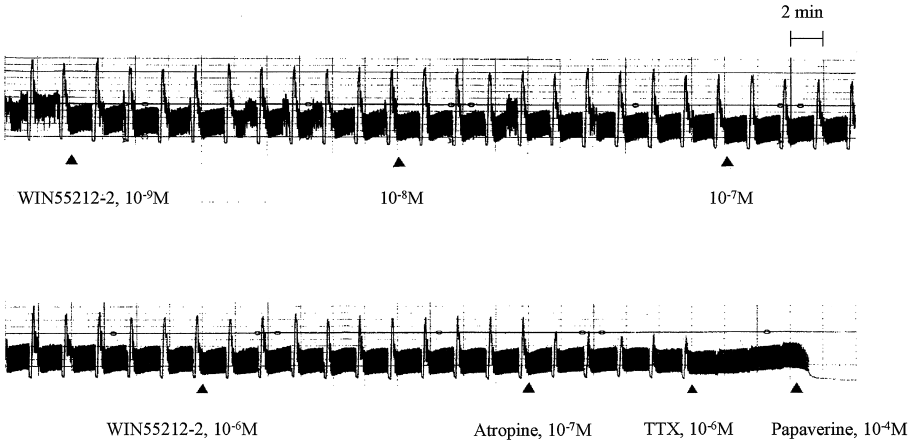

Fig. 6 Representative tracing of the contractile response of guinea pig taenia cacum to electrical field stimulation and its inhibition by WIN55212-2 (10^{-9}～10^{-6}M), atropine (10^{-7}M), tetrodotoxin (TTX, 10^{-6}M), and papaverine (10^{-4}M).

Discussion

In our study, cannabinoids were examined for effects on taenia caecum muscle tension in conscious guinea pigs using a telemetric method, and also for effects on isolated guinea pig taenia caecum. In the in vivo experiment, intraperitoneal administration of cannabinoids immediately decreased longitudinal muscle tension. Cannabinoids did not have direct effects on the isolated taenia caecum in vitro, but they inhibit contractions induced by KCl-related depolarization. Contractions evoked by electrical stimulation also were inhibited by cannabinoids.

Our study is the first to focus on effects of cannabinoids on motility of guinea pig colonic longitudinal muscle in vivo. Monitoring of colonic motility in conscious, unconstrained guinea pigs is capable of evaluating physiologic motility of the colon as well as examining pathophysiology in models of intestinal motor disorders. This is the only method capable of evaluating not only intestinal motility but also muscle tension in the intestinal tract under physiologic conditions. A previous study using this method demonstrated that the resting tonus of guinea pig colonic longitudinal muscle is maintained physiologically at a very high level; this implies the presence of a mechanism responsible for

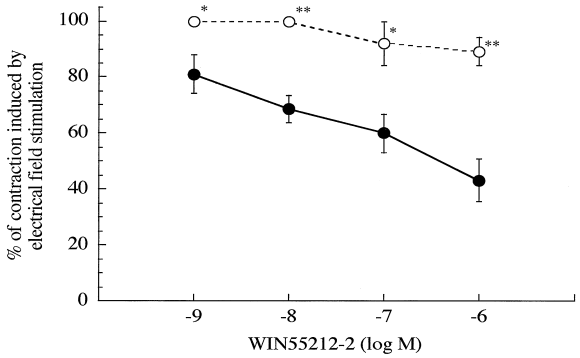

Fig. 7 Inhibitory effect of WIN55212-2 on electrically evoked contractions of strips of guinea pig taenia caecum either alone (control, filled circles) or in the presence of AM281 at 10^{-6} M (open circles). The ordinate shows contraction from electrical stimuli in the presence of drugs as a percentage of contraction induced by electrical field stimulation without drugs. *$p < 0.05$, **$p < 0.01$ compared with the corresponding point in control. Points and bars respectively show the mean ± SEM (n = 3～7 different muscle preparations).

regulation and maintenance of tonus[8]. The results of our experiment using telemetry showed that 2-AG and WIN55212-2 exerted an immediate, potent inhibitory effect on the resting tonus of colonic longitudinal muscle. The inhibitory effect on muscle tension was completely blocked by pretreatment with a CB1 receptor antagonist, and therefore presumably was mediated by CB1 receptors. Colonic propulsion has previously been examined in vivo in mice[6]. Transport of intestinal contents which depepends mainly on contractions of circular muscle, reportedly is inhibited by cannabinoids CB1 receptors[6]. Our study demonstrated that the tension of the guinea pig colonic longitudinal muscle also might be decreased by cannabinoids.

To elucidate the mechanism of the inhibitory effect of cannabinoids on longitudinal muscle tension, we examined effects of cannabinoids in isolated colonic longitudinal muscle. Neither 2-AG nor WIN55212-2 caused any effects on isolated taenia caecum, which suggests that cannabinoids lack direct effects on the longitudinal smooth muscle implying an absence of cannabinoid receptors. This interpretation is supported by a report of immunohistochemical demonstration of cannabinoid receptors in intermuscular and submucosal nerve plexuses, but not in smooth muscle[1,9]. Cannabinoid CB1 receptor agonists apparently can act centrally to modulate colonic motility. Cannabinoids have been reported to have an additional site of action on prejunctional neurons in the guinea pig small intestine[5,10,11].

When effects of cannabinoids were examined in the presence of intestinal smooth muscle contractile agents, neither WIN55212-2 nor 2-AG interrupted the maintenance of contractions caused by ACh, but both inhibited the maintenance of contractions induced by KCl. It has been shown that the ACh-induced contraction of smooth muscle is consequent on the receptor-operated Ca^{2+}-influx and that KCl-induced contraction results from the voltage-dependent Ca^{2+}-influx. The inhibitory effects of WIN55212-2 and 2-AG were observed only in KCl-induced contraction in this study, which suggests that the relaxant action of cannabinoids depends on the muscle membrane potential. The inhibitory effect of WIN55212-2 against maintenance of KCl-induced contractions was not suppressed by a CB1 receptor antagonist or a CB2 receptor antagonist; instead, the inhibition was countered by charybdotoxin, a Ca^{2+}-activated K^+-channel inhibitor. This finding suggested that inhibition of contractions might be related to K^+-channel opening. It has been reported that a Ca^{2+}-activated K^+-current was potentiated by activation of the G-protein-coupled atypical cannabinoid receptor distinct from CB1 or CB2[12]. Anandamide has been also reported to induce relaxation of rat mesenteric artery smooth muscle independently of CB1 receptors[13,14]. There is some other evidence that current cannabinoid receptor classification may be incomplete with the identification of non-CB1 non-CB2 cannabinoid-induced responses[15]. This finding suggests that cannabinoids may have a variety of pharmacologic actions.

However, these effects observed in our in vitro experiments differed from our observations in vivo, where cannabinoids did not change the amplitude of contractions but did decrease tension. This disagreement may be explained by a difference in loaded tension (1 g applied to the isolated taenia

caecum in vitro vs. approximately 30 g in vivo), or by hypothesizing that the tension-reducing effect in vivo is mediated by central CB1 receptors. A finding supporting the latter explanation is that when body temperature was measured simultaneously with examination of intestinal tension, the agonists decreased intestinal tension and body temperature almost simultaneously. In a recent report, hypothermia was induced in rats when WIN55212-2 was administered either intraperitoneally or directly into the preoptic anterior hypothalamus, a finding consistent with involvement of central CB1 receptors[16]. Yet, regulation of intestinal tension by a CNS CB1 receptor mechanism awaits definitive proof.

To determine whether cannabinoids act up on the prejunctional neurons of the taenia caecum, we electrically stimulated guinea pig longitudinal muscle specimens. Atropine decreased the amplitude of electrically evoked contractions, suggesting that the contractions might be related to ACh release from prejunctional cholinergic neuron endings. Since the electrically evoked contractions were suppressed by tetrodotoxin, which blocks action potential propagation in nerve, they may be related to excitatory transmission. The amplitude of the electrical stimulation-induced contractions also was decreased by WIN55212-2, which accordingly WIN55212-2 may have inhibited ACh release from nerve endings via CB receptors on the cholinergic neurons[11] in Auerbach's nerve plexus, located between the colonic longitudinal muscle and the circular muscle. The effect of WIN55212-2 was inhibited by pretreatment with AM281, a specific CB1 receptor antagonist and therefore appears to be mediated by prejunctional CB1 receptors.

Taken together, the results of the in vitro and in vivo experiments suggested that peripheral cannabinoid CB1 receptors in the intestinal nerve plexus may be involved in regulating peristalsis of intestinal tract, while CNS cannabinoid CB1 receptors may be involved in regulating intestinal tonus.

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