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Contractile response to a cannabimimetic eicosanoid, 2-arachidonoylglycerol, of longitudinal smooth muscle from the guinea-pig distal colon in vitro

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Abstract

The effect of 2-arachidonoylglycerol, a cannabimimetic eicosanoid, was studied on mucosa-free longitudinal muscle strips isolated from the guinea-pig distal colon. In the presence of indomethacin (3 μM) and N^G-nitro-L-arginine (100 μM), 2-arachidonoylglycerol (10 nM–10 μM) produced concentration-dependent and tetrodotoxin (1 μM)-sensitive contractions of the longitudinal muscle strips. The contractions were markedly attenuated in the presence of atropine (0.2 μM), and partially by hexamethonium (100 μM) pretreatment. The response to 2-arachidonoylglycerol was mimicked with *N*-arachidonoylethanolamine (anandamide, 0.1–30 μM), another cannabimimetic eicosanoid, but the cannabinoid CB_1/CB_2 receptor agonist, *R*-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3,-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (WIN55,212-2) (0.1–10 μM), and the vanilloid receptor agonist, (all *Z*)-(4-hydroxyphenyl)-5,8,11,14-eicosatetraenamide (AM 404) (10–30 μM), were without effect. The cannabinoid CB_1 receptor antagonist, *N*-piperidino-5-(4-chlorophenyl)-l-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-caroxamide (SR141716A) (1 μM), the cannabinoid CB_2 receptor antagonist, [*N*-[1*S*]-endo-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-l-(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528) (1 μM), and the vanilloid receptor antagonist, capsazepine (10 μM), did not shift the concentration–response curve for 2-arachidonoylglycerol to the right. The contractile action of 2-arachidonoylglycerol was also partially attenuated in the presence of nordihydroguaiaretic acid (10 μM), a lipoxygenase inhibitor. These results indicate that 2-arachidonoylglycerol produces contraction of longitudinal muscle of the guinea-pig distal colon via mainly stimulation of myenteric cholinergic neurones, and that neither cannabinoid CB_1/CB_2 receptors nor vanilloid receptors contributed to the response. The present results suggest the possibility that lipoxygenase metabolites may also contribute, at least in part, to the contractile action of 2-arachidonoylglycerol.

Keywords: 2-Arachidonoylglycerol; Anandamide; Cannabinoid; Colon, guinea-pig

1. Introduction

Cannabinoids have been shown to exert a broad variety of pharmacological actions, including central and peripheral effects, through receptor-mediated mechanisms (Howlett, 1995). Pharmacological and molecular biological studies have identified at least two types of cannabinoid receptors, denoted CB_1 and CB_2 receptors, both coupled to G-proteins (Felder and Glass, 1998). The expression of cannabinoid CB_1 receptors is largely confined to the central nervous system (Matsuda et al., 1990), but recent studies provide evidence for the existence of cannabinoid CB_1 receptors in the enteric nervous system (Pertwee, 2001). Together with the characterization of receptor subtypes, there has been a concerted effort to identify natural endogenous ligands. *N*-arachidonoylethanolamine (anandamide) has been isolated from brain as the first endogenous ligand for cannabinoid receptors (Devane et al., 1992; Devane and Axelrod, 1994). Another possible candidate is 2-arachidonoylglycerol, which has been isolated from brain and intestine (Sugiura and Waku, 2000).

In the guinea-pig ileum, anandamide has been shown to inhibit electrically evoked contractions, probably via presynaptic inhibition of acetylcholine release and this effect was reversed by SR141716A, a cannabinoid CB_1 receptor antagonist (Izzo et al., 1998). A recent report demonstrated

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that anandamide increases basal acetylcholine release via stimulation of vanilloid receptors in the guinea-pig ileum (Mang et al., 2001). However, there is still little information about the pharmacological action of 2-arachidonoylglycerol, a second type of endogenous cannabinoid, on intestinal motility. In the present study, we first demonstrated that 2-arachidonoylglycerol produces a neurogenic contraction of longitudinal smooth muscle of the guinea-pig distal colon. We then tried to assess the mechanisms through which 2-arachidonoylglycerol exerts its contractile effect on the longitudinal smooth muscle.

2. Materials and methods

2.1. Tissue preparation

Male Dunkin–Hartley guinea-pigs, weighing 250–450 g, were anaesthetized with enflurane and bled. A distal colon segment was excised from 6 to 12 cm proximal to the pelvic brim. Strips of mucosa-free longitudinal muscle (1.5 cm long) from the distal colon were prepared as described in a previous study (Kojima and Shimo, 1996). The strips were suspended in the longitudinal direction under a 0.5-g load in 10-ml tissue baths filled with a modified Krebs solution (mM: NaCl 120, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, Na_2EDTA 0.03) at 37 °C and bubbled with 5% CO_2–95% O_2. The modified Krebs solution always contained indomethacin (3 μM) and N^G-nitro-L-arginine (100 μM), to minimize endogenous prostanoid biosynthesis and nitric oxide biosynthesis in response to endocannabinoids. In some experiments, we used mucosa-free circular muscle strips (8 mm long, 4 mm wide) to establish whether endocannabinoids produce a contraction of the circular muscle of the guinea-pig colon. After setting up, the strips were allowed to equilibrate for at least 60 min with renewal of the bathing solution every 15 min. Changes in mechanical activity of the tissue were isotonically recorded on a Nihon Kohden polygraph (RJG-4128) through an isotonic transducer (Nihon Kohden, TD-112S).

2.2. Experimental protocol

Concentration–response curves for agonists were constructed in a cumulative manner. In most cases, one of four preparations served as control and the three others were for the study of agonists in the presence of a set concentration of antagonist. Antagonists were allowed to equilibrate for 30–60 min before the addition of agonists. The size of contractions evoked by agonists was expressed as percentage of the maximum response obtained with carbachol (10 μM) in each preparation. Carbachol was added at the first stage of the experimental protocol. Percentage contractions were plotted as mean values to obtain log concentration–response curves.

2.3. Analysis of data

The values of E_{max} were expressed as percentages of the maximum response to carbachol (10 μM). The values of pEC20 or pEC30 (negative logarithm of molar concentration eliciting 20% or 30% of the maximum contraction) were calculated from individual concentration–response curves, according to Van Rossum (1963). Means ± S.E.M. of n experiments are given throughout the paper. The significance of the difference between mean values was assessed by Student's unpaired t-test, or by Dunnett's multicomparison test when appropriate. Results were considered significant if $P < 0.05$.

2.4. Drugs

The following drugs were used: atropine sulphate, hexamethonium chloride dihydrate (Wako, Osaka, Japan); tetrodotoxin (Sankyo, Tokyo, Japan); (all *Z*)-(4-hydroxyphenyl)-5,8,11,14-eicosatetraenamide (AM 404), capsazepine, indomethacin, N^G-nitro-L-arginine, nordihydroguaiaretic acid (Sigma, St. Louis, MO, USA); anandamide, palmitoylethanolamide, *R*-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3,-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (WIN 55,212-2) mesylate (Tocris Cookson, Bristol, UK); *N*-piperidino-5-(4-chlorophenyl)-l-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-caroxamide (SR141716A) and [*N*-[1*S*]-endo-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-l-(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528), were a kind gift from Dr. Madene Mosse and Dr. Francis Barth (SANOFI-Recherche, Montpellier, France). 2-Arachidonoylglycerol was synthesized in our laboratory. Indomethacin (100 μM) was dissolved in distilled water containing

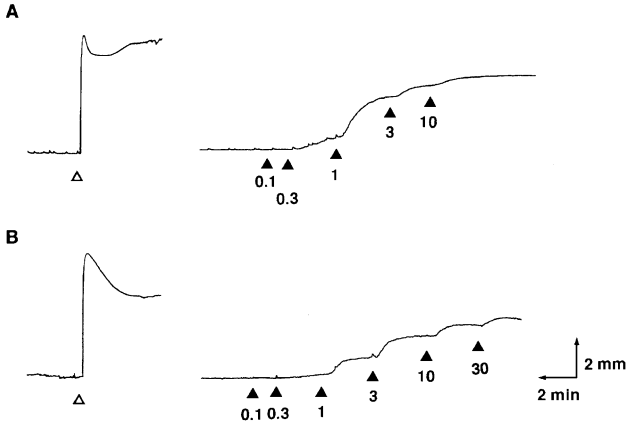

Fig. 1. Typical tracings showing the contractile responses of the mucosa-free longitudinal muscle of guinea-pig isolated distal colon to: (A) 2-arachidonoylglycerol (▲, 0.1–10 μM); (B) anandamide (▲, 0.1–30 μM). In the first stage of the experiments, carbachol (△, 10 μM) was added to obtain the maximal contraction of the colon strips. Vertical calibration shows 2-mm shortening of the tissue, horizontal calibration shows 2 min. Indomethacin (3 μM) and N^G-nitro-L-arginine (100 μM) were present throughout the experiments.

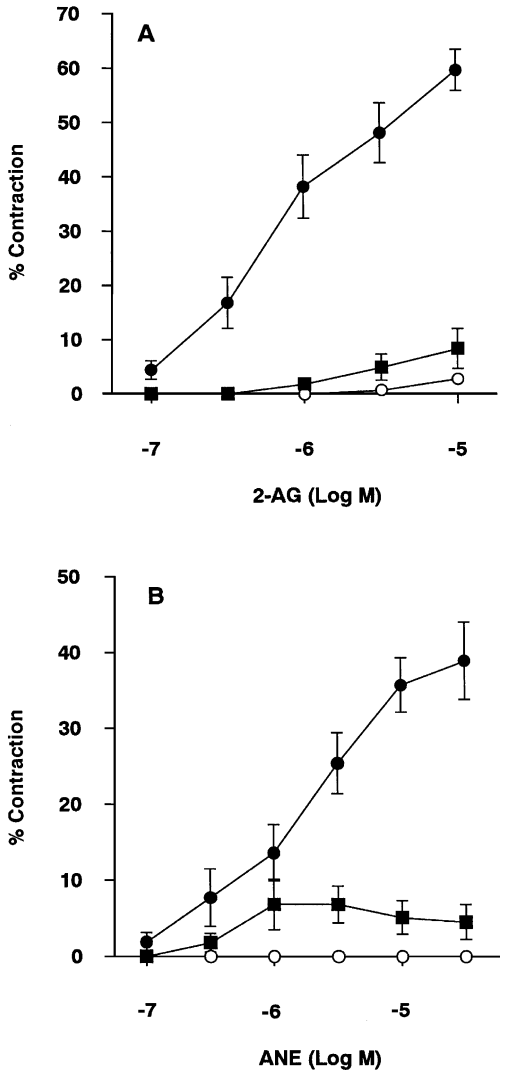

Fig. 2. Concentration–response curves for 2-arachidonoylglycerol (2-AG) (A)- and anandamide (AEA) (B)-evoked contraction of guinea-pig distal colon in the absence (●) and in the presence of 1 μM tetrodotoxin (○) or 0.2 μM atropine (■). Each point represents the mean ± S.E.M. for 6–11 preparations and is a percentage of the maximal contraction in response to 10 μM carbachol. Indomethacin (3 μM) and N^G-nitro-L-arginine (100 μM) were present throughout the experiments.

equimolar concentrations of Na_2CO_3. 2-Arachidonoylglycerol, AM 404, anandamide, capsazepine, palmitoylethanolamide, SR141716A and SR144528 were initially dissolved in 100% dimethylsulfoxide. The vehicles had no effects on the basal tone of the longitudinal muscle. The concentrations mentioned are the calculated final concentrations in the bath solution.

3. Results

3.1. Effects of agonists

In the presence of indomethacin (3 μM) and N^G-nitro-L-arginine (100 μM), 2-arachidonoylglycerol (10 nM–10 μM, $n=11$) produced a sustained contraction of longitudinal muscle from the guinea-pig distal colon, in a concentration-dependent manner. The maximum response obtained at 10 μM amounted to 59.7 ± 3.8% of the carbachol (10 μM)-evoked one and the pEC30 value was 6.06 ± 0.15 (Figs. 1A and 2A). Anandamide (0.1–30 μM, $n=7$) mimicked the 2-arachidonoylglycerol-evoked contraction with a pEC30 value of 5.24 ± 0.26 and a maximum effect at 30 μM, amounting to 38.9 ± 5.1% of the effect of carbachol (10 μM) (Figs. 1B and 2B). The contractile responses to both endocannabinoids were abolished in the presence of tetrodotoxin (1 μM), a nerve conduction blocking agent, and strongly inhibited in the presence of atropine (0.2 μM) (Fig. 2). In contrast, palmitoylethanolamide (0.1–30 μM, $n=4$), a vanilloid receptor agonist, AM 404 (10–30 μM, $n=4$), and a synthetic cannabinoid CB_1/CB_2 receptor agonist, WIN55,212-2 (0.1–10

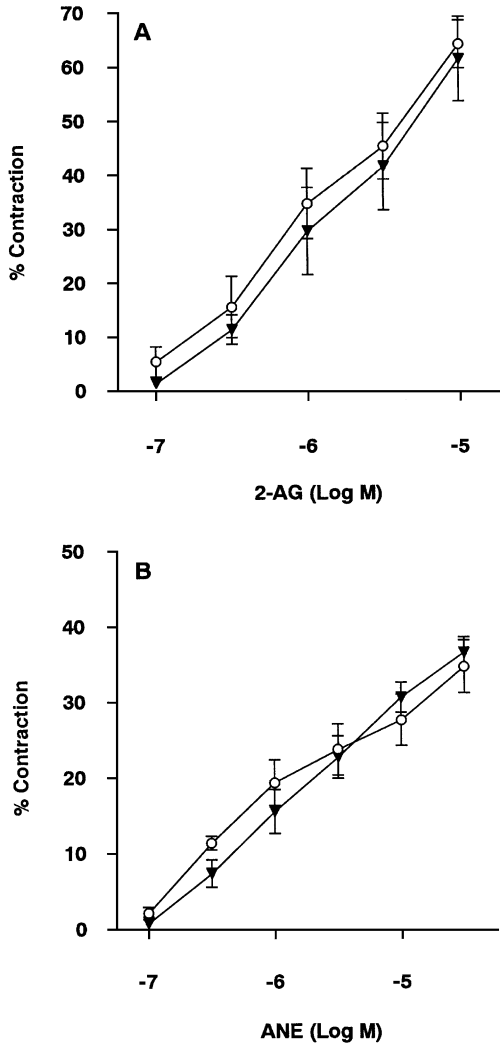

Fig. 3. Concentration–response curves for 2-arachidonoylglycerol (2-AG) (A)- and anandamide (AEA) (B)-evoked contraction of guinea-pig distal colon in the presence of 1 μM SR141716A (○) or 10 μM capsazepine (▼). Each point represents the mean ± S.E.M. for six preparations and is a percentage of the maximal contraction in response to 10 μM carbachol.

Table 1
The potencies of 2-arachidonoylglycerol (2-AG) in guinea-pig colon in the absence and presence of various antagonists

Antagonists		2-AG	
		pEC_{30} (or pEC_{20})	E_{max} (%)
Control		6.06 ± 0.15 (6.30 ± 0.14)[a]	59.7 ± 3.8
SR141716A	(1 μM)	6.05 ± 0.20	64.3 ± 4.4
SR144528	(1 μM)	5.86 ± 0.18	54.4 ± 3.9
Capsazepine	(10 μM)	5.73 ± 0.20	61.6 ± 7.8
Nordihydroguaiaretic acid	(10 μM)	5.57 ± 0.20[a,b]	36.0 ± 4.1[b]
Hexamethonium	(100 μM)	5.61 ± 0.04[b]	40.0 ± 4.9[b]

Listed are pEC_{30} values (or pEC_{20}) and E_{max} values (percentage of the effect of carbachol, 10 μM).
The data are means ± S.E.M. from 6 to 11 preparations. Indomethacin (3 μM) and N^G-nitro-L-arginine (100 μM) were present throughout experiments.
[a] pEC_{20} values.
[b] $P < 0.05$ compared with control.

μM, $n = 4$), failed to produce a contraction of these longitudinal muscle strips.

Neither 2-arachidonoylglycerol (0.1–30 μM, $n = 4$) nor anandamide (0.1–30 μM, $n = 4$) significantly affected the resting tone of circular muscle strips.

3.2. Effects of antagonists

Several antagonists were tested against the 2-arachidonoylglycerol- or anandamide-evoked contractions of the guinea-pig distal colon. Neither SR141716A (1 μM, $n = 6$), a cannabinoid CB_1 receptor antagonist, nor capsazepine (10 μM, $n = 6$), a vanilloid receptor antagonist, caused any significant alteration of the concentration–response curves for 2–arachidonoylglycerol and anandamide (as compared to control curves in Fig. 2) (Fig. 3A,B and Table 1). SR144528 (1 μM, $n = 6$), a cannabinoid CB_2 receptor antagonist, did not significantly affect the concentration–response curve for 2-arachidonoylglycerol (Table 1). In the presence of nordihydroguaiaretic acid (10 μM, $n = 6$), a lipoxygenase inhibitor or hexamethonium (100 μM, $n = 6$), the concentration–response curve for 2-arachidonoylglycerol was shifted rightward with significant depression of E_{max} to 2-arachidonoylglycerol (Table 1).

Nordihydroguaiaretic acid (10 μM, $n = 4$) did not significantly affect the concentration–response curve for acetylcholine (10 nM–30 μM, data not shown).

4. Discussion

The present study has shown that an endogenous cannabimimetic eicosanoid, 2-arachidonoylglycerol, produces concentration-dependent and reproducible contractions of longitudinal muscle of the guinea-pig distal colon in the presence of indomethacin and N^G-nitro-L-arginine. Since tetrodotoxin totally abolished the contractions, this response was due to stimulation of enteric nerves, and not to activation of the release of prostanoids or nitric oxide. The contractile action of 2-arachidonoylglycerol was markedly attenuated by atropine, implicating mediation by acetylcholine release from cholinergic nerves. The 2-arachidonoylglycerol-evoked contraction was partially hexamethonium-sensitive, suggesting that the response is in part mediated by the facilitation of nicotinic cholinergic transmission. Thus, 2-arachidonoylglycerol may activate preganglionic parasympathetic nerve terminals or cholinergic interneurones to release acetylcholine. The neurogenic contraction evoked by 2-arachidonoylglycerol in colon strips was also mimicked by another endogenous cannabimimetic eicosanoid, anandamide, with less potency. A possible explanation for the lesser potency of anandamide may be a greater metabolic inactivation in this tissue. 2-Arachidonoylglycerol has been shown to bind to the cannabinoid CB_1/CB_2 receptors and exhibit a variety of cannabimimetic activities in vitro and in vivo (Sugiura and Waku, 2000). However, the present results suggest that the contractile response to 2-arachidonoylglycerol is not mediated via cannabinoid CB_1/CB_2 receptors, since neither a cannabinoid CB_1 receptor antagonist, SR141716A, nor a cannabinoid CB_2 receptor antagonist, SR144528, shifted the concentration–response curve for 2-arachidonoylglycerol to the right. The lack of agonistic activity of a cannabinoid CB_1/CB_2 receptor agonist, WIN55,212-2, is also an argument against the involvement of cannabinoid CB_1/CB_2 receptors. Further, both 2-arachidonoylglycerol and anandamide failed to contract circular smooth muscle preparations of the guinea-pig distal colon. Thus, the present results support the view that cholinergic neurones innervating longitudinal smooth muscle may express a novel cannabinoid system.

Anandamide has been described as an agonist at the vanilloid receptor, mediating relaxation of guinea-pig basilar artery (Zygmunt et al., 1999) or increase of basal acetylcholine release from guinea-pig ileum (Mang et al., 2001). In the present study, however, a vanilloid receptor antagonist, capsazepine, did not shift the concentration–response curves for 2-arachidonoylglycerol or anandamide to the right. In addition, a potent vanilloid receptor agonist, AM 404 (Zygmunt et al., 2000), failed to contract this preparation. Thus, it seems likely that the vanilloid receptor is not involved in the contractile response of guinea-pig colon to 2-arachidonoylglycerol or anandamide. In this study, we also found that a general lipoxygenase inhibitor, nordihydroguaiaretic acid (Tornhamre et al., 1989), partially attenuates the 2-arachidonoylglycerol-evoked contraction, suggesting mediation by production of lipoxygenase metabolites. It is of course possible that 2-arachidonoylglycerol is being metabolized to lipoxygenase products through the production of arachidonic acid (Sugiura et al., 1997). It has been found recently that lipoxygenase metabolites of anandamide induce contractions of the guinea-pig isolated bronchus (Craib et al., 2001). However, we cannot exclude the

possibility that 2-arachidonoylglycerol itself stimulates the release of lipoxygenase products from guinea-pig distal colon. The nature of lipoxygenase products and the mechanism involved is the subject of ongoing investigations.

In conclusion, our data indicate that 2-arachidonoylglycerol produced a contraction of longitudinal muscle of the guinea-pig distal colon mainly via stimulation of myenteric cholinergic neurones, and that neither cannabinoid CB_1/CB_2 receptors nor vanilloid receptors contributed to the 2-arachidonoylglycerol-evoked contraction. The present results also suggest the possibility that lipoxygenase metabolites may contribute, at least in part, to the 2-arachidonoylglycerol-evoked contraction.

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