British Journal of Pharmacology (1998) 124, 1363–1368
http://www.stockton-press.co.uk/bjp

Excitatory transmission to the circular muscle of the guinea-pig ileum: evidence for the involvement of cannabinoid CB_1 receptors

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1. The effect of cannabinoid drugs has been investigated on cholinergic and non-adrenergic non-cholinergic (NANC) contractile responses to the circular smooth muscle of guinea-pig ileum elicited by electrical field stimulation (EFS).

2 The cannabinoid receptor agonist WIN 55,212-2 (1–1000 nM) and the putative endogenous ligand anandamide (0.1–100 μM) both produced a concentration-dependent inhibition of the cholinergic (9–57% and 1–51% inhibition) and NANC (9–55% and 2–57% inhibition) contractile responses. WIN 55,212-2 and anandamide did not modify the contractions produced by exogenous acetylcholine or substance P.

3 Apamin (30 nM), a blocker of Ca^{2+}-activated K^+ channels, reduced the inhibitory effect of WIN 55,212-2 on cholinergic, but not NANC, contractile response. N^G-nitro-L-arginine methyl ester (100 μM), an inhibitor of nitric oxide synthase, or naloxone (1 μM), an opioid receptors antagonist, did not modify the inhibitory effect of WIN 55,212-2 on both cholinergic and NANC contractions.

4 The inhibitory effects of WIN 55,212-2 and anandamide on both cholinergic and NANC contractile response was competitively antagonized by the cannabinoid CB_1 receptor antagonist SR 141716A (10–1000 nM).

5 In absence of other drugs, SR 141716A (1–1000 nM) enhanced cholinergic (1–45% increase) and NANC (2–38% increase) contractile responses elicited by electrical stimulation, but did not modify the contractions produced by acetylcholine or substance P.

6 It is concluded that activation of prejunctional cannabinoid CB_1 receptors produces inhibition of cholinergic and NANC excitatory responses in the guinea-pig circular muscle. The inhibition of cholinergic (but not NANC) transmission involves activation of apamin-sensitive K^+ channels. In addition, an endogenous cannabinoid ligand could inhibit cholinergic and NANC transmission in the guinea-pig ileal circular muscle.

Keywords: Myenteric plexus; cannabinoids; intestinal motility; anandamide; small intestine

Introduction

It is now well known that cannabinoids can exert their biological functions through receptor-mediated mechanisms (Howlett, 1995; Pertwee, 1997). Two types of cannabinoid (CB) receptors have been identified to date, denoted as the CB_1 receptor, which is located both in the brain and some peripheral tissues (Devane *et al*., 1988; Howlett, 1995) and the CB_2 receptor, which is located in the periphery only (Munro *et al*., 1993). Griffin *et al*. (1997) have demonstrated the presence of specific cannabinoid CB_1 receptors in the myenteric plexus-longitudinal muscle preparation of the guinea-pig small intestine. In this preparation cannabinoid agonists inhibit electrically-evoked contractions (Pertwee *et al*., 1992; 1996b; Coutts & Pertwee, 1997). As these agonists in this preparation do not inhibit the contractions produced by exogenous acetylcholine but decrease the electrically-evoked release of acetylcholine (Coutts & Pertwee, 1997), it is likely that these drugs act prejunctionally to inhibit cholinergic transmission. These responses are reversed by SR 141716A (Coutts & Pertwee, 1997; Pertwee *et al*., 1996b), a selective antagonist of the cannabinoid CB_1 receptors (Rinaldi-Carmona *et al*., 1994). Electrophysiological studies have shown that the cannabinoid receptor agonist WIN 55,212-2, acting as CB_1 receptors, inhibits fast and slow synaptic transmission in myenteric neurones innervating the longitudinal muscle of the guinea-pig ileum (Lòpez-Redondo *et al*., 1997).

From above, it appears that the role of cannabinoid receptors in the intestine have been analysed mostly using longitudinal muscle with myenteric plexus attached. Therefore the present study sought to investigate the effect of cannabinoid drugs on cholinergic and non-adrenergic non-cholinergic (NANC) excitatory transmission in the guinea-pig circular muscle.

Methods

Male guinea-pigs (250–400 g) were killed by asphyxiation with CO_2. Portions of the ileum lying 5–15 cm proximal to the ileocaecal junction were removed quickly and placed in Krebs solution. The ilea were cut, parallel to the circular muscle, as 2–3 mm wide rings (Maggi *et al*., 1993), suspended in 20 ml baths for isolated organs by means of two stainless steel hooks and bathed with warm (37°C), aerated (95% O_2 : 5% CO_2) Krebs solution (composition in mM: NaCl, 119; KCl, 4.75; KH_2PO_4, 1.2; $NaHCO_3$, 25; $MgSO_4$, 1.5; $CaCl_2$ 2.5 and glucose, 11). The mechanical activity of the circular muscle was recorded with an isotonic transducer (load 0.5 g) connected to a 'Gemini' recording apparatus (Ugo Basile, Comerio, Italy). Once mounted in the organ baths, the ileal rings were subjected to an electrical field stimulation (EFS) delivered via electrodes placed around the tissue. Cholinergic and NANC contractions were obtained by EFS (10 Hz for 0.3 s, 100 mA, 0.5 ms pulse duration for cholinergic contractions and 32 Hz for 1 s,

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100 mA, 0.5 ms pulse duration for NANC contractions). In order to block cholinergic and adrenergic components, NANC contractions were obtained in the presence of atropine (1 μM) and guanethidine (3 μM). Stable and reproducible contractions for a time-period of 3 h were obtained with stimulations every 2 min and were expressed as % of contraction produced by KCl 80 mM. This concentration of KCl produced a circular muscle contraction corresponding to total occlusion of the ileal lumen. In preliminary experiments, the effect of hexamethonium (100 μM), tetrodotoxin (100 nM), or SR 140333 (0.1 μM) plus SR 48968 (1 μM) was evaluated on electrically-induced contractions. These compounds were left in contact with the tissue for 20 min (hexamethonium), 10 min (tetrodotoxin) and 30 min (SR 141033 plus SR 48968). During these time-periods, the tissue was stimulated every 2 min.

After stable control contractions evoked by EFS of the cholinergic and NANC nerves had been recorded, the responses were observed in the presence of increasing cumulative concentrations of WIN 55,212-2 (1 – 1000 nM), anandamide (0.1 – 100 μM), SR 141716A (1 – 1000 nM), DAGO (1 – 100 nM) or papaverine (0.1 – 10 μM). The contact time for each concentration was 10 min (15 min for SR 141716A and anandamide). To determine antagonistic activity (pA_2), WIN 55,212-2 (or anandamide) was tested 20 min after SR 141716A (10, 100 and 1000 nM). In some experiments papaverine or DAGO were tested after SR 141716A (1 μM). Since by itself SR 141716A increased the electrically-evoked contractions, the addition of agonists was made after the amplitude of the twitch response had reached a new steady level. This occurred 15 – 20 min after SR 141716A administration.

The effect of WIN 55,212-2 was also evaluated 20 min after N^G-nitro-L-arginine methyl ester (L-NAME 100 μM), apamin (30 nM), naloxone (1 μM) or phentolamine (1 μM). In some experiments DAGO (1 – 100 nM) or papaverine (0.1 – 10 μM) were tested alone or after apamin (30 nM).

When single concentrations were used, these were selected on the basis of previous work (Rand & Li, 1993; Maggi & Giuliani, 1996; Pertwee *et al*., 1996b; Izzo *et al*., 1997). The concentration of apamin (30 nM) was chosen on the basis of laboratory experience: lower concentrations of apamin (10 nM) produced a weaker effect, while higher concentrations (100 nM) were as active as 30 nM. In some experiments, contractions were produced by exogenous acetylcholine (0.01 – 10 μM) or substance P (0.1 – 100 nM), concentration-response curves being constructed non-cumulatively with a concentration-cycle of 15 min and the effect of WIN 55,212-2 (1 μM), anandamide (100 μM) and SR 141716A (1 μM) evaluated. The interval between addition of acetylcholine or substance P was 15 min (10 min with WIN 55,212-2).

To verify that the method used detected only circular muscle activity, in preliminary experiments ($n=7$) the ileal rings were exposed to prostaglandin (PG) $F_{1\alpha}$, an agonist known to contract ileal longitudinal, but not circular muscle (Bennett *et al*., 1975). $PGF_{1\alpha}$ (40 μM) did not contract the ileal rings, but produced sustained contractions of the guinea-pig ileum set up to record longitudinal muscle activity (data not shown).

Drugs

Drugs used were: [D-Ala^2,N-methyl-Phe^4,Gly^5-ol]enkephalin (DAGO), acetylcholine chloride, apamin, atropine sulphate, guanethidine sulphate, hexamethonium chloride, naloxone hydrochloride, N^G-nitro-L-arginine methyl ester hydrochloride, phentolamine hydrochloride, prostaglandin $F_{1\alpha}$, substance P acetate, tetrodotoxin (Sigma, Milan, Italy), arachidonylethanolamide (amandamide), WIN 55,212-2 R(+)-[2,3-dihydro-5methyl-3-[4-morpholinyl)methyl]pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl] (1-naphthalenyl) methanone mesylate (RBI, Milan, Italy). SR 141716A (N-piperidin-1-yl)-5-(-4-chloropenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride), SR 140333 (S)1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl) piperidin-3-yl]ethyl}-4-phenyl-1-azoniabicyclo[2,2,2]octane chloride and SR 48968 (S)-N-methyl-N[4-(4-acetylamono-4-phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl]benzamide hydrochloride were a gift from Dr Daniel Aubert and Madelein Mossè (SANOFI-Recherche, Montpellier, France). WIN 55,212-2 and SR 141716A were dissolved in DMSO, anandamide in ethanol and SR 140333 in DMSO/water (50%, v/v). The other drugs were dissolved in distilled water. All drugs were added in volumes less than 0.1% of the bath volume. DMSO or ethanol (0.05%) had no effect on the responses under study. Vehicles of SR 141716A, apamin, L-NAME, naloxone or phentolamine did not modify the concentration-response to WIN 55,212-2 on electrically-induced contractions.

Statistical analysis

Results are given as mean ± s.e.mean (or 95% confidence limits of the IC_{50} values). Comparisons between two sets of data were made by Student's *t* test for paired data. When multiple comparisons against a single control were made, analysis of variance was used, followed by the modified *t*-test according to Bonferroni. Analysis of variance (two way with replication) was used to compare different cumulative concentration-effect curves. Probability less than 0.05 was regarded as significant. The concentration of cannabinoids that produced 30% inhibition of the twitch response (IC_{30}) was used to characterize their potency. IC_{30} values [geometric mean ± 95% confidence limits (c.l.)] were determined by probit analysis.

Competitive antagonism was quantified as the ratio of equi-active molar concentration. These values were estimated at the level of the half-maximal response. Antagonist activity (pA_2) was estimated with the Schild analysis of these data (Arunlakshana & Schild, 1959).

Results

Cholinergic and NANC contractions produced by EFS

Electrical stimulation (10 Hz for 0.3 s, 100 mA, 0.5 ms pulse duration) of the ileal circular muscle gave a contractile response which was 25 ± 4% of the contraction produced by 80 mM KCl. These contractions were not altered by the ganglion blocking drug hexamethonium (100 μM, $n=5$), but were abolished by atropine (1 μM, $n=5$) or tetrodotoxin (100 nM, $n=4$). The twitch responses were thus mediated primarily by electrical depolarization of postganglionic cholinergic nerves. Apamin (30 nM) and L-NAME (100 μM) increased (98 ± 6% increase, $P<0.01$ and 35 ± 6% increase, $P<0.05$ respectively, $n=5$) the cholinergic electrically-evoked contractions, while naloxone (1 μM) or phentolamine (1 μM) had no significant effect.

In the presence of atropine (1 μM) and guanethidine (3 μM) electrical field stimulation (32 Hz for 1 s, 100 mA, 0.5 ms pulse duration) of the ileal circular muscle produced contractile responses of the circular muscle preparation which were 29 ± 4% of the contraction produced by 80 mM KCl. These contractions were unaffected by hexamethonium (100 μM), but strongly reduced by tetrodotoxin (89 ± 4%

inhibition) and by a combination of SR 1400333 (0.1 μM) and SR 48968 (1 μM), antagonists of the receptors NK_1 and NK_2 respectively (85±4% inhibition, $n=5$). Thus the NANC contractile response is mediated mainly by the release of tachykinins from postganglionic neurones.

Apamin (30 nM) and L-NAME (100 μM), *per se*, increased the NANC contractions (122±7% increase $P<0.01$, $n=5$ and 65±5% increase $P<0.01$, $n=5$), while naloxone (1 μM) was without significant effect.

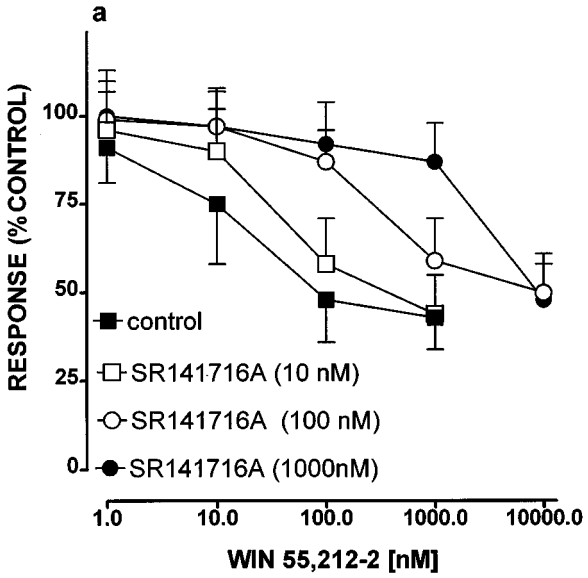
Effect of cannabinoids drugs on cholinergic and NANC contractions produced by EFS

WIN 55,212-2 (1–1000 nM) decreased the amplitude of the cholinergic [IC_{30} (95% c.l.): 48 (18, 81) nM]- and NANC [IC_{30} (95% c.l.): 51 (22, 89) nM]-evoked contractions in a concentration-dependent manner (Figure 1). Statistical significance ($P<0.05$–0.01) for both cholinergic and NANC contractions was achieved for concentrations of 100 and 1000 nM. The inhibitory effect was competitively antagonized (Figure 1) by SR 141716A (10–1000 nM). The pA_2 values were 8.08±0.30 (Schild Slope: 0.97) for cholinergic contractions and 8.22±0.01 (Schild Slope: 0.99) for NANC contractions. SR 141716A (1 μM) did not modify the inhibitory effect of DAGO (% of control response after DAGO: 1 nM, 87±11; 3 nM, 65±12; 10 nM, 42±11; 30 nM, 20±13; 100 nM, 9±12; % of control response after DAGO plus SR 141716A: 1 nM, 95±13; 3 nM, 80±9; 10 nM, 40±14; 30 nM, 10±5; 100 nM, 6±5) or papaverine (% of control response after papaverine; 0.1 μM, 98±13; 0.3 μM 90±15; 1 μM, 65±12; 3 μM, 34±14; 10 μM, 5±15; % of control response after papaverine plus SR 141716A: 0.1 μM, 94±12; 0.3 μM, 91±13; 1 μM, 78±13; 3 μM, 25±11; 10 μM, 3±10).

Given alone SR 141716A markedly increased cholinergic- and NANC-induced contractions, an effect which was significant ($P<0.05$) starting from 10 nM (Figure 2).

The inhibitory effect of WIN 55,212-2 on both cholinergic and NANC contractions was unaffected by naloxone (1 μM) or L-NAME (100 μM) (Figure 3a and b). By contrast apamin (30 nM) significantly ($P<0.05$) reduced the inhibitory effect of WIN 55,212-2 on cholinergic (Figure 3a) but not on NANC contractions (Figure 3b). Apamin (30 nM) did not modify the inhibitory effect of DAGO (1–100 nM) or papaverine (0.1–10 μM) on cholinergic contractions (data not shown). In addition, the inhibitory effect of WIN 55,212-2 on cholinergic contractions was unaffected by phentolamine (1 μM) (data not shown).

Anandamide (0.1–100 μM) also reduced the amplitude of the cholinergic [IC_{30} (95% c.l.): 54.8 (39.2, 95.3) μM] and NANC [IC_{30} (95% c.l.): 51.8 (37.7, 88.0) μM] contractions of ileal circular muscle (Figure 4). Statistical significance ($P<0.05$–0.01) for both cholinergic and NANC contractions was achieved for concentrations of 10 and 100 μM. The



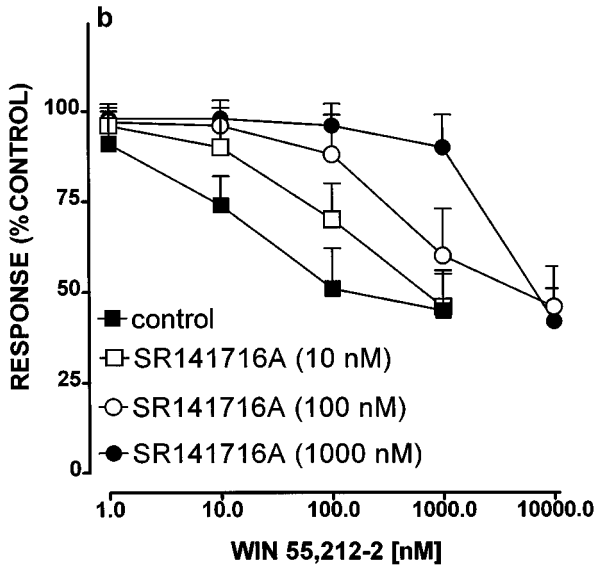


Figure 1 Inhibitory effect of WIN 55,212-2 on cholinergic (a) and NANC (b) contractile response produced by EFS in the circular muscle of the guinea-pig ileum alone (control) or in the presence of SR 141716A at concentration of 10 nM, 100 nM and 1000 nM. The ordinates show the percentage of control response. Each point represents the mean of 5–6 experiments; vertical lines show s.e.mean.

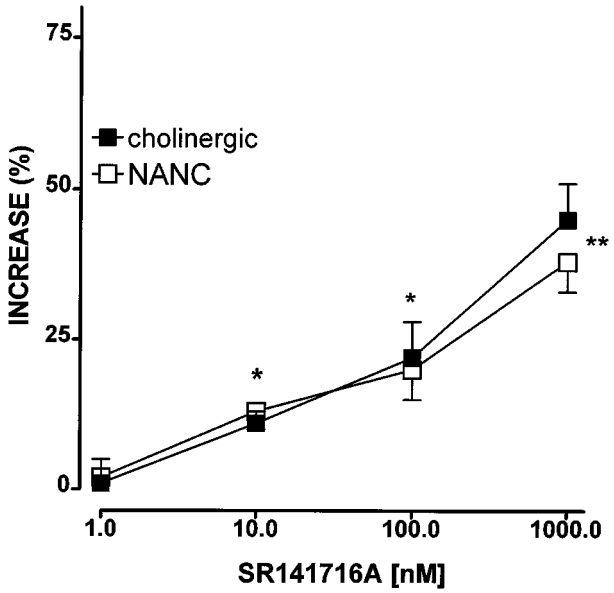


Figure 2 Augmenting effect of SR 141716A on cholinergic and NANC contractions produced by EFS in the circular muscle of the guinea-pig ileum. The ordinates show the percentage increase above the control response. Each point represents the mean of 12–14 experiments; vertical lines show s.e.mean. *$P<0.05$ and **$P<0.01$ *vs* corresponding control.

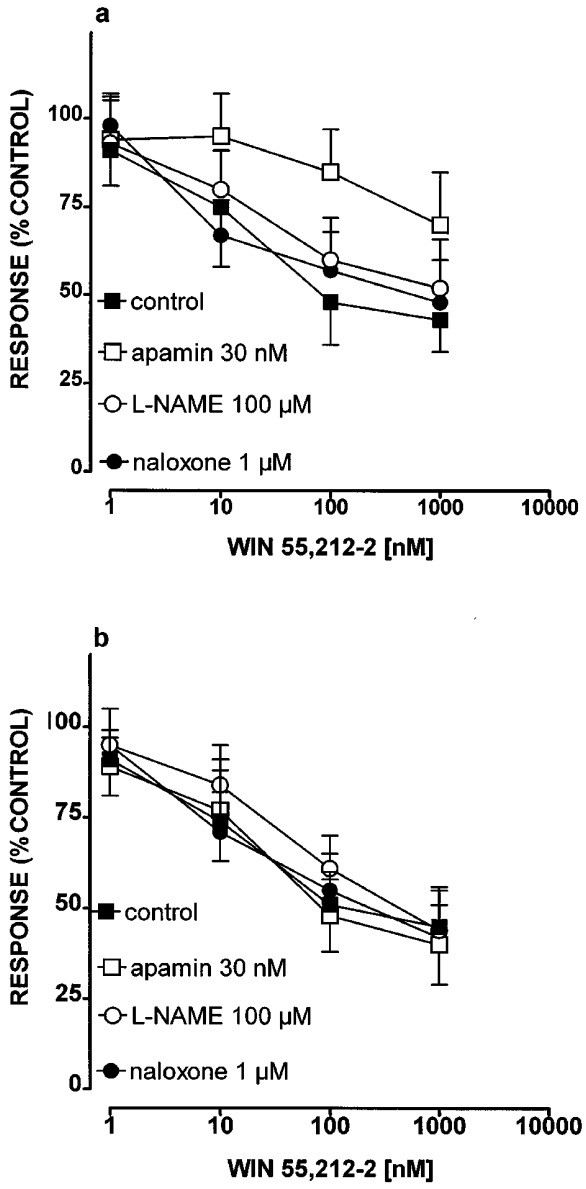


Figure 3 Inhibitory effect of WIN 55,212-2 on cholinergic (a) and NANC (b) contractions produced by EFS in the circular muscle of the guinea pig ileum alone (control) or in presence of apamin (30 nM), L-NAME (100 μM) or naloxone (1 μM). The ordinates show the percentage of control response. Each point represents the mean of five experiments; vertical lines show s.e.mean.

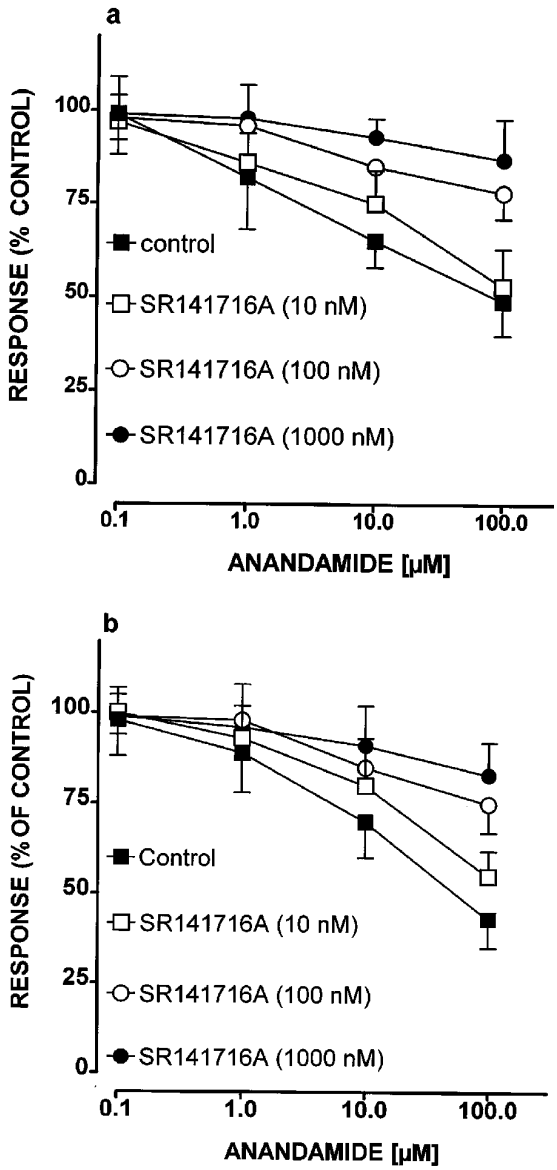


Figure 4 Inhibitory effect of anandamide on cholinergic (a) and NANC (b) contractile response produced by EFS in the circular muscle of the guinea-pig ileum alone (control) or in the presence of SR 141716A at concentration of 10 nM, 100 nM and 1000 nM. The ordinates show the percentage of control response. Each point represents the mean of five experiments; vertical lines show s.e.mean.

inhibitory effect was antagonized (Figure 4) by SR 141716A (10–1000 nM). The pA_2 values were 8.21 ± 0.17 (Schild Slope: 0.99) for cholinergic contractions and 8.06 ± 0.14 (Schild Slope: 0.99) for NANC contractions. WIN 55,212-2 (1 μM), anandamide (100 μM) or SR 141716A (1 μM) did not modify significantly the contractions evoked by exogenous acetylcholine (0.01–10 μM) or substance P (0.1–100 nM) (data not shown).

Discussion

Contraction of the circular muscle is dominant in peristalsis (Kosterlitz & Lees, 1964); thus studies on the action of cannabinoids on neuromuscular transmission in the circular muscle could be crucial to understanding their role in regulating intestinal motility. Previous investigators have studied the role of cannabinoid receptors on cholinergic transmission in the guinea-pig longitudinal muscle (Pertwee *et al.*, 1996b; Coutts & Pertwee, 1997). Our data have shown that activation of cannabinoid CB_1 receptors results in inhibition of excitatory cholinergic and NANC tramission to the circular muscle. Conversely blockade of the CB_1 receptors produces enhancement of cholinergic and NANC contractile responses evoked by EFS, raising the possibility that an endogenous cannabinoid could be released under the experimental conditions.

We have shown that WIN 55,212-2, a selective cannabinoid agonist (Compton *et al.*, 1992), and anandamide, an endogenous ligand of these receptors (Devane *et al.*, 1992) can produce a concentration-related inhibition of the electrically-evoked cholinergic and NANC response of the guinea-pig isolated circular muscle. Inhibition of ileal contractions appears to involve prejunctional mechanisms, as

the two ligands did not alter smooth muscle response to exogenous acetylcholine or substance P. These observations are in line with findings from previous experiments with guinea-pig whole ileum (Layman & Milton, 1971) or strips of myenteric plexus-longitudinal muscle (Pertwee *et al*., 1992; 1996a,b).

We have found that WIN 55,212-2 is more active than anandamide in inhibiting electrically-evoked contractions. However, compared to other studies in the guinea-pig small intestine (IC_{50}: 5.54 nM for WIN 55,212-2 and 8.82 μM for anadamide) (Pertwee *et al*., 1995; 1996b), in the present study WIN 55,212-2 and anandamide were found less active. The use of different preparations (circular muscle with mucosa *vs* longitudinal muscle without mucosa), vehicles (DMSO or ethanol *vs* Tween 80) frequency and intensity of stimulation (10 Hz at 100 mA *vs* 0.1 Hz at supramaximal voltage) and other experimental conditions could explain this discrepancy. However, in rat hippocampal slices, other workers have found that WIN 55,212-2 inhibited electrically-evoked acetylcholine release (Gifford & Ashby, 1996) and long-term potentiation (Terranova *et al*., 1995) with an IC_{50} of 30 nM and more than 1 μM respectively.

Several endogenous substances could be released by WIN 55,212-2 and therefore be involved in this inhibitory response. Opioids act on receptors located on neurons within the myenteric plexus, depress the firing of myenteric neurons, inhibit the release of excitatory neurotransmitters and reduce the nerve-mediated contractions of the circular muscle (Kromer, 1988). Nitric oxide (NO) is contained in enteric neurons and it exerts an inhibitory modulatory role on cholinergic and NANC transmission (Brookes, 1993). Noradrenaline, released from post postganglionic sympathetic nerves, acts presynaptically or prejunctionally to prevent acetylcholine release (Wood, 1987). However, it is unlikely that cannabinoids act by releasing NO, opioids or noradrenaline as the NO synthase inhibitor L-NAME, the opioid antagonist naloxone or the α-adrenoceptor antagonist phentolamine failed to modify the inhibitory effect of WIN 55,212-2.

Apamin blocks small conductance Ca^{2+}-dependent K^+ channels in the intestinal smooth muscle (Banks *et al*., 1979). Apamin-sensitive inhibitory transmission is responsible for the fast inhibitory junction potential evoked by transmural nerve stimulation in the circular muscle of the guinea-pig ileum (Crist *et al*., 1992). ATP, or a related purine, has been proposed to be the transmitter of enteric inhibitory motoneurons and evidence suggest that ATP mediates the apamin-sensitive mode of transmission to the circular muscle of the guinea-pig small intestine (Costa *et al*., 1986; Crist *et al*., 1992). We have demonstrated that apamin reduced the depressant effect of WIN 55,212-2, without modifying the inhibitory effect of papaverine and DAGO, indicating that this agonist probably activates apamin-sensitive inhibitory nerves. Consistent with these results Welch *et al*. (1995) have demonstrated that apamin attenuates Δ^9-tetrahydrocannabinol-induced antinociception in mice. However, in contrast to cholinergic transmission, the inhibitory effect of WIN 55,212-2 on NANC responses was unaffected by apamin, indicating that activation of K^+ channels does not play a role. The reason for this discrepancy is still a matter of investigation.

The inhibitory action of both WIN 55,212-2 and anandamide was competitively antagonized by SR 141716A indicating an involvement of CB_1 receptors. The pA_2 values for SR 141716A correlate well with previous studies in the mouse isolated vas deferens (Rinaldi-Carmona *et al*., 1994), bladder (Pertwee & Fernando, 1996), retina (Schlicker *et al*., 1996) and guinea pig myenteric plexus-longitudinal muscle preparation (Coutts & Pertwee, 1997).

In the absence of other drugs, SR 141716A, which is a selective CB_1 antagonist at concentration lower than 1 μM (Rinaldi-Carmona *et al*., 1995), increased the amplitude of cholinergic and NANC electrically-evoked contractions, suggesting that the guinea-pig ileum can itself produce a cannabinoid receptor agonist that has an inhibitory effect on cholinergic and NANC transmission. Our results also exclude a sensitization of smooth muscle myofilaments as SR 141716A did not potentiate the contractions produced by exogenous acetylcholine or substance P. Anandamide, a putative endogenous cannabinoid (Devane *et al*., 1992), decreased electrically-induced contractions, an effect counteracted by SR 141716A. However it is unlikely that anandamide is the endogenous cannabinoid released under our experimental conditions, as this compound has been detected only in the brain (Devane *et al*., 1992). Another possible candidate is 2-arachidonylglycerol, which has already been found in the intestine (Mechoulam *et al*., 1995). Others have found that SR 141716A increased electrically-evoked twitch responses of mouse isolated vas deferens (Pertwee *et al*., 1996a), urinary bladder (Pertwee & Fernando, 1996) and guinea-pig myenteric plexus (Pertwee *et al*., 1996b). In addition, SR141716A facilitates the release of neurotransmitters from rat superfused retinal discs (Schlicker *et al*., 1996), rat hippocampal slices (Gifford & Ashby, 1996) and guinea-pig myenteric plexus (Coutts & Pertwee, 1997).

In summary, we have shown that prejunctional CB_1 receptors are able to modulate cholinergic and NANC contractile responses in the guinea-pig circular muscle. Activation of cannabinoid CB_1 receptors inhibits cholinergic and NANC excitatory response; the inhibitory effect on cholinergic (but not on NANC) tranmission involves activation of apamin-sensitive K^+ channels. Blockade of cannabinoid CB_1 receptors produces an increase in cholinergic and NANC excitatory response indicating that the guinea-pig circular muscle motility could be tonically inhibited by an endogenous cannabinoid system.

This work was supported by CNR, Murst and Enrico and Enrica Sovena Foundation (Roma). We wish to thank Miss Emilia Nocerino for her help.

References

ARUNLAKSHANA, A.O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonist. *Br. J. Pharmacol. Chemother.,* **14,** 153–161.

BANKS, B.E.C., BROWN, C., BURGESS, G.M., BURNSTOCK, G., CLARET, M., COCKS, T.M. & JENKINSON, D.H. (1979). Apamin blocks certain neurotransmitter-induced increases in potassium permeability. *Nature,* **282,** 415–417.

BENNETT, A., ELEY, K.G. & STOCKLEY, H.L. (1975). The effects of prostaglandins on guinea-pig isolated intestine and their possible contribution to muscle activity and tone. *Br. J. Pharmacol.,* **54,** 197–204.

BROOKES, S.J.H. (1993). Neuronal nitric oxide in the gut. *J. Gastroenterol. Hepatol.,* **8,** 590–603.

COMPTON, D.R., GOLD, L.H., WARD, S.J., BALSTER, R.L. & MARTIN, B.R. (1992). Aminoalkylindole analogs: cannabimimetic activity of a class of compounds structurally distinct from Δ^9-tetrahydrocannabinol. *J. Pharmacol. Exp. Ther.,* **263,** 1118–1126.

COSTA, M., FURNESS, J.B. & HUMPHREYS, C.M.S. (1986). Apamin distinguishes two types of relaxation mediated by enteric nerves in the guinea-pig gastrointestinal tract. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **332,** 79–88.

COUTTS, A.A. & PERTWEE, R.G. (1997). Inhibition by cannabinoid receptor agonists of acetylcholine release from the guinea-pig myenteric plexus. *Br. J. Pharmacol.,* **121,** 1557–1566.

CRIST, J.R., HE, X.D. & GOYAL, R.K. (1992). Both ATP and the peptide VIP are inhibitory neurotransmitters in guinea-pig ileum circular muscle. *J. Physiol.,* **447,** 119–131.

DEVANE, W.A., DYSARZ, F.A., JOHNSON, M.R., MELVIN, L.S. & HOWLETT, A.C. (1988). Determination and characterization of a cannabinoid receptor in rat brain. *Mol. Pharmacol.,* **34,** 605–613.

DEVANE, W.A., HANUS, L., BREUER, A., PERTWEE, R.G., STEVENSON, L.A., GRIFFIN, G., GIBSON, D., MANDELBAUM, A., ETINGER, A. & MECHOULAM, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science,* **258,** 1946–1949.

GIFFORD, A.N. & ASHBY, C.R. (1996). Electrically evoked acteylcholine release from hippocampal slices is inhibited by the cannabinoid receptor agonist, WIN 55212-2, and is potentiated by the cannabinoid antagonist, SR 141716A. *J. Pharmacol. Exp. Ther.,* **277,** 1431–1436.

GRIFFIN, G., FERNANDO, S.R., ROSS, R.A., MCKAY, N.G., ASHFORD, M.L.J., SHIRE, D., HUFFMAN, J.W., YU, S., LAINTON, J.A.H. & PERTWEE, R.G. (1997). Evidence for the presence of CB_2-like cannabinoid recepotrs on peripheral nerve terminals. *Eur. J. Pharmacol.,* **339,** 53–61.

HOWLETT, A.C. (1995). Pharmacology of cannabinoid receptors. *Annu. Rev. Pharmacol. Toxicol.,* **35,** 607–634.

IZZO, A.A., MASCOLO, N., COSTA, M. & CAPASSO, F. (1997). Effect of papaverine on synaptic transmission in the guinea-pig ileum. *Br. J. Pharmacol.,* **121,** 768–772.

KOSTERLITZ, H.W. & LEES, G.M. (1964). Pharmacological analysis of intrinsic intestinal reflexes. *Pharm. Rev.,* **16,** 301–339.

KROMER, W. (1988). Endogenous and exogenous opioids in the control of gastrointestinal motility and secretion. *Pharmacol. Rev.,* **40,** 121–162.

LAYMAN, J.M. & MILTON, A.S. (1971). Some action of Δ^1-tetrahydrocannabinol and cannabinol at cholinergic junctions. *Br. J. Pharmacol. Proc. Suppl.,* **40,** 553P.

LOPEZ-REDONDO, F., LEES, G.M. & PERTWEE, R.G. (1997). Effects of cannabinoid receptor ligands on electrophysiological properties of myenteric neurones of the guinea-pig ileum. *Br. J. Pharmacol.,* **122,** 330–384.

MAGGI, C.A. & GIULIANI, S. (1996). Evidence that tachykinin NK_2 receptors modulate resting tone in the rat isolated small intestine. *Br. J. Pharmacol.,* **118,** 1262–1268.

MAGGI, C.A, PATACCHINI, R., MEINI, S. & GIULIANI, S. (1993). Nitric oxide is the mediator of tachykinin NK_3 receptor-induced relaxation in the circular muscle of the guinea-pig ileum. *Eur. J. Pharmacol.,* **240,** 45–50.

MECHOULAM, R., BEN-SHABAT, S., HANUS, L., LIGUMSKY, M., KAMINSKI, N.E., SCHATZ, A.R., GOPHER, A., ALMOG, S., MARTIN, B.R., COMPTON, D.R., PERTWEE, R.G., GRIFFIN, G., BAYEWITCH, M., BARG, J. & VOGEL, Z. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to a cannabinoid receptor. *Biochem. Pharmacol.,* **50,** 83–90.

MUNRO, S., THOMAS, K.L. & ABU-SHAAR, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature,* **365,** 61–65.

PERTWEE, R.G. (1997). Pharmacology of cannabinoid CB_1 and CB_2 receptors. *Pharmacol. Ther.,* **74,** 129–180.

PERTWEE, R.G. & FERNANDO, S.R. (1996). Evidence for the presence of cannabinoid CB_1 receptors in mouse urinary bladder. *Br. J. Pharmacol.,* **118,** 2053–2058.

PERTWEE, R.G., FERNANDO, S.R., GRIFFIN, G., ABADJI, V. & MAKRIYANNIS, A. (1995). Effect of phenylmethylsulphonyl fluoride on the potency of anandamide as an inhibitor of electrically-evoked contractions in two isolated tissue preparations. *Eur. J. Pharmacol.,* **272,** 73–78.

PERTWEE, R.G., FERNANDO, S.R., GRIFFIN, G., RYAN, W.L., RAZDAN, R.K., COMPTON, D.R. & MARTIN, B.R. (1996a). Agonist-antagonist characterization of 6′-cyanohex-2′-yne-Δ8-tetrahydrocannabinol in two isolated tissue preparations. *Eur. J. Pharmacol.,* **315,** 195–201.

PERTWEE, R.G., FERNANDO, S.R., NASH, J.E. & COUTTS, A.A. (1996b). Further evidence for the presence of cannabinoid CB_1 receptors in guinea-pig small intestine. *Br. J. Pharmacol.,* **118,** 2199–2205.

PERTWEE, R.G., STEVENSON, L.A., ELRICK, D.B., MECHOULAM, R. & CORBETT, A.D. (1992). Inhibitory effects of certain enantiomeric cannabinoids in the mouse vas deferens and the myenteric plexus preparation of guinea-pig small intestine. *Br. J. Pharmacol.,* **105,** 980–984.

RAND, M.J. & LI, C.G. (1993). Modulation of acetylcholine-induced contractions of the rat anococcygeus muscle by activation of nitrergic nerves. *Br. J. Pharmacol.,* **110,** 1479–1482.

RINALDI-CARMONA, M., BARTH, F., HEAULME, M., ALONSO, R., SHIRE, D., CONGY, C., SOUBRIE', P., BRELIERE, J.-C. & LE FUR, G. (1995). Biochemical and pharmacological characterization of SR141716A, the first potent and selective brain cannabinoid receptor antagonist. *Life Sci.,* **56,** 1941–1947.

RINALDI-CARMONA, M., BARTH, F., HEAULME, M., SHIRE, D., CALANDRA, B., CONGY, C., MARTINEZ, S., MARUANI, J., NELIAT, G., CAPUT, D., FERRARA, P., SOUBRIE, P., BRELIERE, J.-C. & LE FUR, G. (1994). SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.,* **350,** 240–244.

SCHLICKER, E., TIMM, J. & GOTHERT, M. (1996). Cannabinoid receptor-mediated inhibition of dopamine release in the retina. *Naunyn-Schmiedelberg's Arch. Pharmacol.,* **354,** 791–795.

TERRANOVA, J.-P., MICHAUD, J.-P., LE FUR, G. & SOUBRIE', P. (1995). Inhibition of long-term potentiation in rat hippocampal slices by anandamide and WIN55212-2: reversal by SR141716A, a selective antagonist of CB_1 cannabinoid receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **352,** 576–579.

WELCH, S.P., DUNLOW, L.D., PATRIC, G.S. & RAZDAN, R.K. (1995). Characterization of anandamide- and fluoroanandamide-induced antinociception and cross-tolerance to delta 9-THC after intrathecal administration to mice: blockade of delta 9-THC-induced antinociception. *J. Pharmacol. Exp. Ther.,* **273,** 1235–1244.

WOOD, J.D. (1987). Physiology of the enteric nervous system. In *Physiology of the Gastrointestinal Tract*. ed Johnson, L.R. pp. 67–110. New York: Raven Press.

(Received April 8, 1998
Accepted April 27, 1998)