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# Modulation of the release of endogenous γ-aminobutyric acid by cannabinoids in the guinea pig ileum

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**Abstract**

Interactions between cannabinoid $CB_1$ and GABA receptors and ligands were investigated in the myenteric plexus-longitudinal muscle of the guinea pig ileum. Electrically evoked contractions of the myenteric plexus-longitudinal muscle were inhibited by the cannabinoid receptor agonist CP55,940 ((−)-*cis*-3-[2-Hydroxy-4-(1,1-dimethylheptyl) phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol), the $GABA_B$ receptor agonist, baclofen (4-amino-3-(chlorophenyl) butanoic acid), or exogenous GABA. Electrically evoked contractions of the myenteric plexus-longitudinal muscle were also inhibited by the addition of the GABA releasing agent ethylenediamine. CP55,940 (1 nM) or the endogenous cannabinoid anandamide (arachidonyl ethanolamide, 1 μM) reduced the inhibition produced by ethylenediamine, while in contrast, anandamide (10 μM) significantly increased the inhibition produced by ethylenediamine. The results suggest that while there is no interaction between cannabinoid $CB_1$ and $GABA_B$ receptors in the myenteric plexus-longitudinal muscle of the guinea pig, cannabinoid $CB_1$ receptor stimulation reduces the ethylenediamine-evoked GABA release. In addition, anandamide at higher concentrations also potentiates the inhibitory effect of ethylenediamine at least partly by stimulating vanilloid receptors. 

*Keywords:* Ileum; (Guinea pig); Cannabinoid $CB_1$ receptor; GABA (γ-aminobutyric acid) release; Ethylenediamine; Anandamide

## 1. Introduction

The enteric nervous system possesses a multitude of receptor types. In the ileum myenteric plexus of the guinea pig, these include the cannabinoid $CB_1$ receptors (Pertwee et al., 1996) and GABA receptors (Giotti et al., 1983). Activation of either of these receptors inhibits electrically evoked contractions in the myenteric plexus-longitudinal muscle preparation by reducing the release of acetylcholine. Cannabinoid $CB_1$ and $GABA_B$ receptors are both members of the superfamily of seven transmembrane spanning region, $G_i/G_o$ protein coupled receptors and can inhibit the release of acetylcholine by activating inwardly the rectifying $K^+$ channels, and reducing the opening of $Ca^{2+}$ channels (Cherubini and North, 1984; Mackie et al., 1995). The presence of a GABA synthesizing enzyme, glutamic acid decarboxylase (Jessen et al., 1979), together with the presence of endogenous GABA and its $Ca^{2+}$-dependent release (Kerr and Krantris, 1983), suggests that GABA is a neurotransmitter in the ileum of the guinea pig and is involved in the modulation of intestinal motility (Krantis and Kerr, 1981). Cannabinoid $CB_1$ receptor stimulation also modulates intestinal motility (Heinemann et al., 1999), although the physiological role of cannabinoids in gut function is far from established.

There is a growing evidence for the interaction between cannabinoid and other receptor systems. Recent work has demonstrated that cannabinoid receptor activation inhibits GABAergic transmission in cultured hippocampal neurons (Irving et al., 2000), which may be the mechanism of the antinociceptive effects of cannabinoid compounds. Activation of cannabinoid $CB_1$ receptors was shown to reduce the GABAergic inhibitory postsynaptic currents in rat corpus striatum, with the mechanism likely being the inhibition of presynaptic GABA release (Szabo et al., 1998). Other work has shown that cannabinoid receptor stimulation in striatonigral GABAergic neurons inhibits GABA-uptake (Romero et al., 1998).

Ethylendiamine is a useful compound when investigating the GABA transmission in the ileum of the guinea pig. While

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ethylenediamine has been shown to displace [$^3$H]GABA by binding to $GABA_A$ receptors in rat brain preparations (Hill, 1985), it has been clearly demonstrated to directly stimulate the release of endogenous GABA in the ileum of the guinea pig, without displaying any GABA-mimetic properties (Kerr and Ong, 1984). This was established by using the GABA release inhibitor 3-mercaptopropionic acid (Fan et al., 1981) to (a) prevent the release of [$^3$H]GABA from preloaded ileum by ethylenediamine and (b) prevent ethylenediamine-induced GABA-mimetic responses without affecting those elicited by GABA or its analogues.

Transmitter interactions could also occur at the agonist level. The endogenous cannabinoid receptor agonist, anandamide (arachidonyl ethanolamide), and the vanilloid receptor agonist, capsaicin, evoked similar inward currents in HEK293 cells transfected with vanilloid VR1 receptors (Smart et al., 2000). The currents evoked by these ligands were blocked by a vanilloid receptor antagonist but were unaffected by cannabinoid receptor antagonists, suggesting that anandamide acts as a full agonist at vanilloid receptors in these cells. The aim of the present study was to examine the possible interactions between cannabinoid $CB_1$ receptors and $GABA_B$ receptors and to investigate the effect of cannabinoid compounds on evoked GABA release and uptake in the myenteric plexus-longitudinal muscle in the ileum of the guinea pig.

## 2. Materials and methods

### 2.1. Preparation of the myenteric plexus-longitudinal muscle

The myenteric plexus-longitudinal muscle was dissected from the small intestine of Dunkin Hartley guinea pigs (450–550 g) using the method described by Paton and Zar (1968). Tissues were immersed in Krebs solution maintained at 37 °C and supplied with 95% $O_2$ and 5% $CO_2$. The Krebs solution contained (in mM): NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, $KH_2PO_4$ 1.2, $NaHCO_3$ 25, glucose 11.1, $CaCl_2$ 2.5.

A strip of 4 cm of myenteric plexus-longitudinal muscle was mounted in a 30 ml organ bath under an initial tension of 0.5 g. Electrical field stimulation (110% of the voltage which produced a maximal contraction, 0.5 ms duration and 0.1 Hz frequency), applied through two parallel platinum plate electrodes fixed at either side of the longitudinal strip, was generated using a Multistim D330 System stimulator (Digitimer, UK). Contractions of the myenteric plexus-longitudinal muscle were recorded by a Dynamometer UF1 isometric transducer (Pioden Controls, UK), connected via a Conditioning Unit (Techman, UK) to a MX216 Chartrecorder (Electromed, UK).

### 2.2. Experimental design

The myenteric plexus-longitudinal muscle was electrically stimulated for the entire duration of the experiment and it was noted that the size of the contractions increased over the first 3 h, after which the size of the contractions was stable. Hence, no drugs were added until 3 h had elapsed. Concentration–response curves to cannabinoid receptor agonists were performed cumulatively, with 20 min between additions. Only one concentration–response curve was generated per tissue. Previous work has shown that the cannabinoid compounds cannot be removed from the organ bath by washing (Pertwee et al., 1992). In competition studies, the cannabinoid $CB_1$ receptor ligand, SR141716 (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4,-di-chlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride) was added 30 min before the subsequent addition of any other compounds. At the end of every experiment involving cannabinoids, the organ baths were washed with dilute hydrochloric acid, absolute ethanol, and copious amounts of distilled water to ensure the complete removal of any residual cannabinoids. Concentration–response relationships to GABA receptor agonists were also acquired cumulatively, the inhibition was allowed to plateau before the next dose was added ($\sim$20 s). GABA receptor antagonists were added 5 min before the subsequent addition of compounds. GABAergic compounds are readily removed from the organ bath by washing with Krebs solution, so several concentration–response relations could be obtained from a single tissue, with washing between each set. The response at each concentration from repeated concentration–response relations were averaged to form a single *n* number. For experiments using the GABA-releasing agent ethylenediamine (Kerr and Ong, 1984), a period of 2 h was allowed between administrations for the tissue to fully resynthesize GABA after the first application of ethylenediamine. All experiments were performed with relevant parallel controls to ensure that no time dependent changes occurred.

### 2.3. Drugs

CP55,940, anandamide and capsazepine were obtained from Tocris UK. SR141716 and SR144528 (*N*-[(1*S*)-endo-1,3,3-trimethyl bicyclo[2.2.1]-heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methoxybenzyl)-pyrazole-3-carboxamide) were a kind gift from Sanofi Recherche, France. All cannabinoid drugs were dissolved in ethanol, shielded from light and kept at −20 °C, with the exception of anandamide which was supplied dissolved at a concentration of 10.1 mg/ml in a soya oil/water (1:4) emulsion, shielded from light and kept at 4 °C. GABA, baclofen (4-amino-3-(chlorophenyl) butanoic acid), (−)bicuculline methiodide, and ethylenediamine were obtained from Sigma, UK, dissolved in distilled water, and kept at 4 °C. CGP54626A ([*S*-(*R*',*R*')]-[3-[[1-3(,4-dichlorophenyl)ethyl]amino]-2-hydroxypropyl ](cyclohexylmethyl)phosphonic acid) was a kind gift from Dr. W. Froestl (Novartis), dissolved in distilled water, and kept at 4 °C. Tiagabine (3-Piperidinecarboxylic acid, 1-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-nipecotic acid, hydrochloride)

was a kind gift from Dr. A. D'Halluin-Sulzer (Sanofi) dissolved in distilled water, and kept at 4 °C.

### 2.4. Analysis of data

Each value is expressed as the mean ± standard error of the mean of at least six experiments. The effects of cannabinoid and GABA receptor agonists are expressed as the percentage inhibition of contraction. This was calculated by comparing the amplitude of the electrically evoked contraction immediately prior to adding any compound to the amplitude of the contraction at the maximal effect of the compound. The concentration, which produced 50% of the maximum response ($EC_{50}$), was calculated for certain experiments together with 95% confidence limits using the GraphPAD Prism statistical software (GraphPAD Software, CA, USA). Significant difference between two concentration–response curves was calculated using a symmetrical (2 + 2) dose parallel line assay (Colquhoun, 1971) using responses to pairs of agonist concentrations on the steepest part of the curve. In none of these analyses did the pairs of curves deviate significantly from parallelism ($P > 0.05$). Mean values of two sets of data were compared using Student's unpaired *t*-test, a $P$ value $< 0.05$ being taken as significant. Where appropriate, a one-way analysis of variance (ANOVA) test was performed, followed by a post-hoc Dunnett's test to calculate the significant differences between multiple test groups or a post hoc Newman–Keuls to calculate significant differences between all groups of data.

## 3. Results

### 3.1. Effects of cannabinoid $CB_1$ and $GABA_B$ receptor stimulation on the electrically evoked contractions of myenteric plexus-longitudinal muscle

Electrically evoked contractions of the myenteric plexus-longitudinal muscle were inhibited by the cannabinoid receptor agonist CP55,940 in a concentration-dependent manner ($EC_{50} = 25$ nM, 95% confidence limits of 19 and 28 nM, $n = 12$, Fig. 1). The inhibition was slow in onset, reached a maximal plateau within 15 min after each dose, and could not be reversed by washing with Krebs. CP55,940 produced a maximum inhibition of 85 ± 4%. The specific cannabinoid $CB_1$ receptor ligand, SR141716 100 nM, caused a significant rightward shift ($EC_{50}$ for CP55,940 increased to 130 nM, 95% confidence limits of 115 and 138 nM $n = 12$, Fig. 1). The endogenous cannabinoid receptor agonist anandamide (100 μM) also inhibited the electrically evoked contractions of the myenteric plexus-longitudinal muscle. The maximum inhibition produced by 100 μM anandamide (36 ± 10%, data not shown) was prevented by pretreatment with SR141716 (100 nM). Addition of SR141716 (100 nM) alone increased the size of the electrically evoked contractions by 20 ± 10% ($n = 12$).

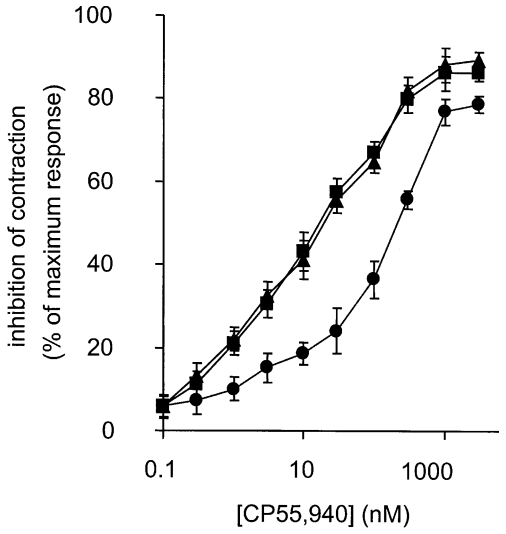


Fig. 1. Effect of the cannabinoid receptor agonist, CP55,940 on electrically evoked contractions of the guinea-pig myenteric plexus-longitudinal muscle. The mean concentration–response curves for the cannabinoid receptor agonist, CP55,940 (■, $n = 12$) and CP55,940 in the presence of the selective cannabinoid $CB_1$ receptor ligand, SR141716 (●,100 nM , $n = 12$), or the selective $GABA_B$ antagonist, CGP54626A (▲, 200 nM, $n = 6$).

The specific $GABA_B$ receptor agonist, baclofen, and exogenous GABA also inhibited the electrically evoked contractions in a concentration-dependent manner (Fig. 2), $EC_{50} = 7$ μM for baclofen (95% confidence limits of 5 and 10 μM) and 6 μM for GABA (95% confidence limits of 4 and 11 μM). The specific $GABA_B$ receptor ligand, CGP54626A (20 nM) produced significant rightward shifts to the effects of baclofen and GABA. The $EC_{50}$ for baclofen increased to 30 μM (95% confidence limits of 22 and 37 μM, $n = 6$, Fig. 2A), and GABA to 33 μM (95% confidence limits of 23 and 41 μM, $n = 6$, Fig. 2B). Baclofen and GABA produced similar maximum inhibitions of 37 ± 3% and 35 ± 4%. CGP54626A (20 nM) had no effect on the size of the electrically evoked contractions ($n = 6$), however, a higher concentration of CGP54626A (200 nM) significantly increased the electrically evoked contractions by 25 ± 5% ($n = 6$). The inhibition produced by GABA was partly transient in nature and the contraction amplitude returned to 85 ± 5% of its original size after 184 ± 15 s. A typical chart recorder trace, illustrating the time course of this reversal of inhibition, is shown in Fig. 5A. CGP54626A (20 nM, $n = 6$) had no effect on the concentration–response curve to CP55,940 (Fig. 1) and SR141716 (100 nM, $n = 6$) had no effect on the concentration–response curve to baclofen (Fig. 2A) or GABA (Fig. 2B).

Preincubation of the myenteric plexus-longitudinal muscle with CP55,940 at a concentration of 1 or 3 nM (20 min, $n = 6$, Fig. 2) evoked significant leftward shifts in the concentration–effect curves and a significant increase in the maximum inhibitions to baclofen. The $EC_{50}$ for baclofen decreased to 1 μM for 1 nM (95% confidence limits of 0.3

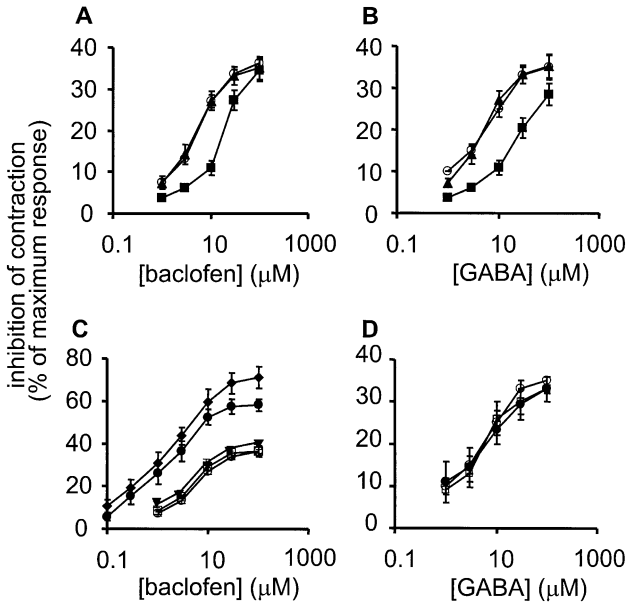


Fig. 2. Effect of GABA receptor stimulation on electrically evoked contractions of the guinea-pig myenteric plexus-longitudinal muscle. (A) The mean concentration–response curves for the $GABA_B$ receptor agonist, baclofen (○), and the effect of preincubation with the $GABA_B$ antagonist on this, CGP54626A (■, 20 nM, 5 min, $n=6$) or the selective cannabinoid $CB_1$ receptor ligand, SR141716 (▲, 100 nM, 30 min, $n=6$). (B) The mean concentration–response curves for the GABA (○), and the effect of preincubation with CGP54626A (■, 20 nM, 5 min, $n=6$) or SR141716 (▲, 100 nM, 30 min, $n=6$). (C) The effect of a 20-min pretreatment with the cannabinoid receptor agonists CP55,940 (●, 1 nM, $n=6$; ◆, 3 nM, $n=6$) or anandamide (□, 10 μM, $n=6$), and a combination of CP55,940 (1 nM) and SR141716 (100 nM, ▼, $n=6$) on the concentration–response curves for baclofen (○). (D) The effect of a 20-min pretreatment with CP55,940 (●, 1 nM, $n=6$; ◆, 3 nM, $n=6$) and anandamide (□, 10 μM, $n=6$), and a combination of CP55,940 (1 nM) and SR141716 (30 min before CP55,940, 100 nM, ▼, $n=6$) on the concentration–response curves for GABA (○). N.B. the *y*-axis scale on panel (C) is twice the size of those in the other panels.

and 2.3 μM) and 0.7 μM for 3 nM (95% confidence limits of 0.1 and 2.2 μM, Fig. 2). CP55,940 (1 nM) had no significant effect on the size of the electrically evoked contractions, whereas 3 nM non-significantly reduced the contraction size by 10 ± 5%. SR141716 (100 nM, $n=6$, Fig. 2C) prevented the effect of 1 nM CP55,940. The endogenous cannabinoid, anandamide (20 min, 10 μM, $n=6$), had no effect on the size of the electrically evoked contractions and did not evoke any shift in the concentration–effect curve to baclofen (Fig. 2C). Pretreatment with CP55,940 (20 min, 1 nM, $n=6$, Fig. 2D) or anandamide (20 min, 10 μM, $n=6$, Fig. 2D) did not cause any shift in the concentration–effect curve to exogenous GABA. Pretreatment with any of the cannabinoid receptor ligands had no effect on the time course of the transient inhibition by GABA (data not shown).

The GABA-uptake inhibitor, tiagabine 1 μM (Fraser et al., 1999; Jackson et al., 1999), had no effect on the size of the electrically evoked contractions but greatly increased the duration of the inhibitory effect of GABA (there was no reversal of inhibition). A sample trace of this effect is shown in Fig. 5A. The inhibition was reversed by CGP54626A (200 nM).

### 3.2. Effect of ethylenediamine on electrically evoked contractions of myenteric plexus-longitudinal muscle

Electrically evoked contractions of the myenteric plexus-longitudinal muscle were inhibited by the addition of a GABA-releasing agent, ethylenediamine (Kerr and Ong, 1984) 1 mM, producing 21 ± 2% inhibition ($n=12$) and 10 mM producing 48 ± 3% inhibition ($n=12$, Fig. 3). A typical chart recorder trace of the effect of a 10 mM ethylenediamine is shown in Fig. 5. The inhibition by ethylenediamine, like that of GABA, was transient in nature and contraction amplitude returned to 83 ± 4% of the inhibition after 191 ± 13 s. Tiagabine (1 μM) also sustained the inhibition produced by ethylenediamine (there was no reversal), an effect that was also reversed by CGP54262A (200 nM). The inhibition produced by ethylenediamine (1 mM) was abolished in the presence of CGP54626A (200 nM, $P<0.001$, $n=6$), while the inhibition by 10 mM ethylenediamine was significantly reduced to 15 ± 3% by CGP54626A (200 nM, $P<0.01$, $n=6$). A second administration of ethylenediamine (10 mM, after a 2-h interval), produced no significant difference in the magnitude of inhibition (46 ± 4%, $n=12$) compared to the first administration.

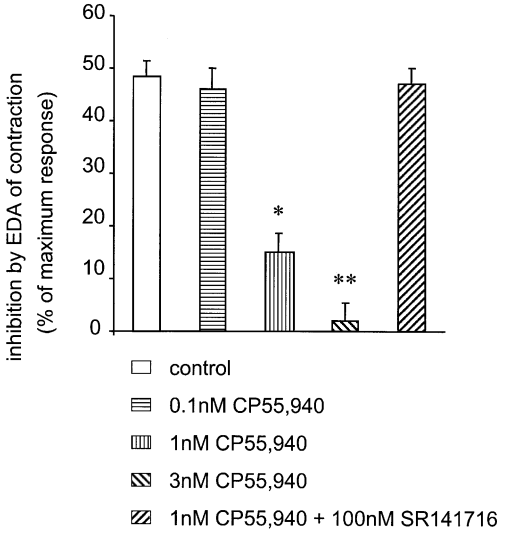


Fig. 3. The effect of the cannabinoid receptor agonist CP55,940 on the inhibition of electrically evoked contractions of guinea-pig myenteric plexus-longitudinal muscle by ethylenediamine (10 mM). The effect of CP55,940 (0.1, 1, 3 nM, $n=6$) on the inhibition of the electrically evoked contractions by ethylenediamine (10 mM), and the effect of SR141716 (100 nM, $n=6$) on the response produced by 1 nM CP55,940. Vertical lines indicate standard error of the mean. *, ** indicate a significant difference from control using a one-way ANOVA followed by a post-hoc Dunnett's test of $P<0.05$ and $P<0.01$, respectively.

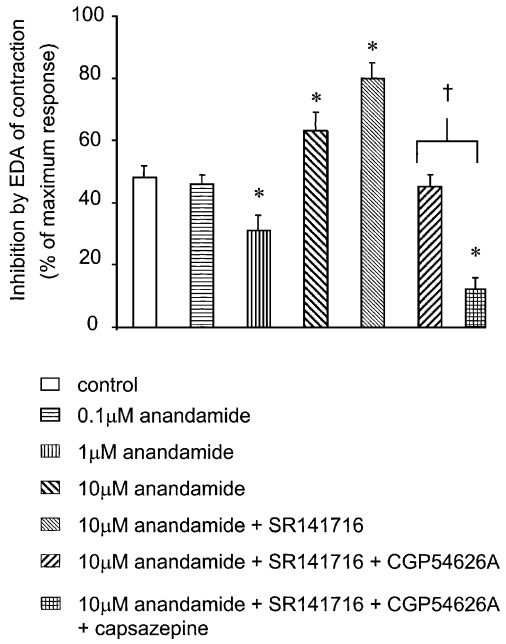


Fig. 4. The effect of the cannabinoid receptor agonist anandamide on the inhibition of electrically evoked contractions of guinea-pig myenteric plexus-longitudinal muscle by ethylenediamine (10 mM). The effect of anandamide (0.1, 1, 10 μM, $n=6$) on the inhibition of the electrically evoked contractions by ethylenediamine (10 mM), and the effect of SR141716 (100 nM, $n=6$), SR141716 (100 nM) and CGP54626A (200 nM, $n=6$), and SR141716 (100 nM) with CGP54626A (200 nM) and capsazepine (1 μM, $n=6$) on the response produced by 10 μM anandamide. Vertical lines indicate the standard error of the mean. * indicates a significant difference from control using a one-way ANOVA followed by a post-hoc Dunnett's test of $P<0.05$. Furthermore, † indicates a significant difference of $P<0.05$ as shown by a post-hoc Newman–Keuls test.

### 3.3. Effect of cannabinoid compounds on the inhibition of electrically evoked contractions by ethylenediamine

Pretreatment of the myenteric plexus-longitudinal muscle with CP55,940 produced a concentration-dependent reduction in the inhibition produced by ethylenediamine (10 mM, Fig. 3). A typical chart recorder trace of the effect of 1 nM CP55,940 is shown in Fig. 5B. The reduction in ethylenediamine-evoked inhibition by CP55,940 (1 nM) was prevented by the presence of SR141716 (100 nM, $n=6$, Fig. 3). Pretreatment with CP55,940 had no effect on the time course of the ethylenediamine induced inhibition (contraction amplitude returned to 86 ± 5% at 195 ± 12 s). None of the concentrations of CP55,940 significantly reduced the size of the electrically evoked contractions, although the 3 nM concentration did reduce the mean contraction size by 10 ± 5%.

Preincubation of the myenteric plexus-longitudinal muscle with anandamide (20 min, 1 μM, $n=6$) significantly reduced the inhibition produced by ethylenediamine to 31 ± 5% (10 mM, $P<0.05$, Fig. 4). However, preincubation with 10 μM anandamide (20 min, $n=12$) significantly *increased* the inhibition produced by ethylenediamine to 62 ± 6% (10 mM, $P<0.05$, Fig. 4). A typical chart recorder trace of this experiment is shown in Fig. 5C. None of the concentrations of anandamide used affected the size of the electrically evoked contractions. However, the contractions could be inhibited using a 100 μM anandamide by 30 ± 8%, which had a slow onset and could not be reversed by washing with Krebs. The increase in the ethylenediamine-evoked inhibition caused by anandamide (10 μM) was further enhanced by SR141716 to 80 ± 5% (1 μM, $n=6$). This large inhibition produced by the combination of ethylenediamine, anandamide (10 μM), and SR141716 was

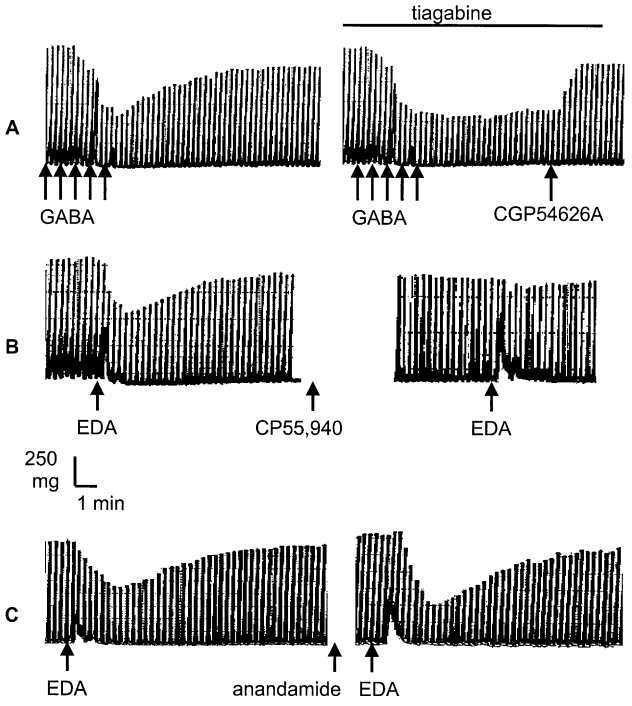


Fig. 5. Sample chart recorder traces of the effects of GABA and cannabinoid compounds related compounds on the electrically evoked contractions of the guinea-pig myenteric plexus-longitudinal muscle. (A) The effect of a GABA concentration response relation on electrically evoked contractions of the guinea-pig myenteric plexus-longitudinal muscle. Upward deflection denotes an electrically evoked contraction. The initial part of the trace illustrates the inhibitory effects of GABA, arrows indicating the point at which the cumulative concentrations of GABA (0.1–100 μM) were administered. The second part of the trace shows the effect the GABA uptake inhibitor, tiagabine, (1 μM) has on the same cumulative concentrations of GABA. CGP54626A (200 nM) was administered at the end of the experiment at the point marked with an arrow. (B) The effect of CP55,940 (1 nM). Initial part of trace indicates the effect of ethylenediamine (10 mM) on electrically evoked contractions, second part denotes the effect CP55,940 had on a subsequent administration of ethylenediamine on electrically evoked contractions. A 2 h period was left between ethylenediamine treatments, and CP55,940 was added 20 min before the second ethylenediamine treatment. (C) The effect of anandamide (10 μM). Initial part of trace indicates the effect of ethylenediamine (10 mM) on electrically evoked contractions, second part denotes the effect anandamide had on a subsequent administration of ethylenediamine on electrically evoked contractions. A 2 h period was left between ethylenediamine treatments, and anandamide was added 20 min before the second ethylenediamine treatment. Upward deflection denotes an electrically evoked contraction.

reduced to 45 ± 4% by CGP54626A (200 nM, $n = 6$, Fig. 4). This remaining inhibition produced by ethylenediamine in the presence of anandamide, SR141716, and CGP54626A was significantly reduced to 11 ± 3% by the vanilloid receptor antagonist capsazepine (1 μM, $n = 6$, Fig. 4). Capsazepine itself had no effect on the inhibition produced by ethylenediamine ($n = 6$, data not shown). The cannabinoid $CB_2$ receptor ligand, SR144528 (1 μM) had no effect on the increased inhibition produced by ethylenediamine, anandamide (10 μM), and SR141716. Pretreatment with anandamide had no effect on the transient effect of ethylenediamine, contraction amplitude returned to 85 ± 6% after 189 ± 14 s.

Pretreatment with the specific cannabinoid $CB_1$ receptor ligand, SR141716 (1 μM) or the specific cannabinoid $CB_2$ receptor ligand, SR144528 (1 μM) had no effect on the size or time to reversal of the inhibition of the electrically evoked contractions evoked by ethylenediamine (10 mM, data not shown).

All experiments were repeated in the presence of the specific $GABA_A$ receptor antagonist, bicuculline (8 μM, preincubated for 5 or 15 min), and no differences in the results were observed at any point. Vehicle controls (ethanol) were performed for each experiment and had no effect on any response observed (data not shown).

## 4. Discussion

Our results confirm that the activation of cannabinoid $CB_1$ or $GABA_B$ receptors by their appropriate agonists leads to the inhibition of electrically evoked contractions of the myenteric plexus-longitudinal muscle of the guinea pig. This is thought to occur through the reduction in release of acetylcholine (Giotti et al., 1983; Coutts and Pertwee, 1997). The $EC_{50}$ values of the compounds used to reduce electrically evoked contractions were similar to those published in previous work (Giotti et al., 1983), with the exception of the $EC_{50}$ value for CP55,940 (Pertwee et al., 1996), which was four-fold higher in our experiments. A 10-fold concentration of anandamide was required to mimic the inhibitory effects seen by CP55,940 compared to previous work, although this may be due to the lack of an amidase inhibitor which was used to reduce anandamide metabolism in the previous study (Pertwee et al., 1995). The inhibitory effects of cannabinoid and GABAergic compounds were antagonised by compounds specific for the cannabinoid $CB_1$ and $GABA_B$ receptor. The concentration of antagonists used were the same as those previously shown to be selective for these receptors (Bittiger et al., 1993; Pertwee et al., 1996). The results confirm the presence of prejunctional cannabinoid $CB_1$ and $GABA_B$ receptors in the myenteric plexus-longitudinal muscle.

Recent work has illustrated interactions between cannabinoid and other receptor systems (Welch and Eads, 1999; Smart et al., 2000) but this was not seen in the present study of the $GABA_B$ receptors. Antagonism of the $GABA_B$ receptor did not affect the concentration–response curves to the cannabinoid receptor agonist, CP55,940 and antagonism of the cannabinoid $CB_1$ receptor had no effect on the concentration–response curves to the $GABA_B$ receptor agonist, baclofen. Activation of cannabinoid $CB_1$ receptors by CP55,940 evoked a leftward shift in the concentration–response curve to baclofen, accompanied by an increase in the maximum inhibition obtained. While the higher concentration of CP55,940 used produced an inhibition of the electrically evoked contractions, baclofen was still able to produce a further inhibition on top of this that was equal to its maximal inhibitory ability in the absence of CP55,940. This combined inhibitory effect of simultaneous cannabinoid $CB_1$ and $GABA_B$ receptor stimulation suggests that they reduce the electrically evoked contractions of the myenteric plexus-longitudinal muscle through separate pathways. Although the combined effects of baclofen and CP55,940 are very clear, the same combined inhibitory effects are not observed between baclofen and anandamide, and furthermore, the same effect is not mimicked between exogenous GABA and anandamide or CP55,940. The reason for this is unclear. The possibility exists that while baclofen and CP55,940 are synthetic compounds, GABA and anandamide are endogenous compounds which are susceptible to uptake and metabolism masking any possible combined effects.

Ethylenediamine was shown to inhibit the electrically evoked contractions of the myenteric plexus-longitudinal muscle. The specific $GABA_B$ receptor antagonist, CGP 54626A blocked this inhibition, which strongly suggests that ethylenediamine inhibits electrically evoked contractions by releasing endogenous GABA, activating $GABA_B$ receptors. This confirms the previous work that described the ability of ethylenediamine to directly stimulate the release of endogenous GABA from the small intestine of the guinea pig (Kerr and Ong, 1984). CP55,940 concentration-dependent reduced the inhibition evoked by ethylenediamine. However, the single concentration of CP55,950 used produced less inhibition of the electrically evoked contractions than the equivalent concentration used in the construction of the cumulative concentration–response curves. This may be a result of the myenteric plexus-longitudinal muscle being exposed to cannabinoids for a longer duration in the cumulative experiments. SR141716 entirely reversed the CP55,940 induced reduction in the inhibition evoked by ethylenediamine, suggesting that the cannabinoid effect is mediated by cannabinoid $CB_1$ receptors. Only a single concentration of SR141716 was used as its effects on CP55,940 in the MLPM have been thoroughly described previously (Pertwee et al., 1996). SR141716 by itself has no effect on the inhibition produced by ethylenediamine, which suggests that there is no tonic release of endogenous cannabinoids in the myenteric plexus-longitudinal muscle. SR141716 itself did increase the size of the electrically evoked contractions, but this may be due to its inverse-agonist effect (Maclennan et al., 1998), rather than an

antagonism of endogenous cannabinoids. Conversely, CGP 54626A did display the ability to significantly increase the size of the electrically evoked contractions when applied alone at a high concentration, suggesting that there is a tonic release of endogenous GABA.

A low concentration of anandamide also significantly reduced the inhibition produced by ethylenediamine. The effect of anandamide and CP55,940 shows that the activation of cannabinoid $CB_1$ receptor can prevent ethylenediamine-induced GABA release. This effect of anandamide and CP55,940 is in accordance with the observation that cannabinoid $CB_1$ receptor stimulation in rat corpus striatum can inhibit presynaptic GABA-release (Szabo et al., 1998). GABA-release by electrical stimulation is $Ca^{2+}$-dependent (Kerr and Krantis, 1983). GABA-release by ethylenediamine may also be a $Ca^{2+}$-dependent event and the reduction in intracellular $Ca^{2+}$ following the cannabinoid $CB_1$ receptor stimulation due to the inhibition of voltage-gated $Ca^{2+}$ channels (Mackie et al., 1995), may account for the reduction in ethylenediamine-evoked GABA-release following pretreatment with CP55,940.

Uptake mechanisms for GABA have been proposed in the myenteric plexus-longitudinal muscle (Kerr and Krantis, 1983). Tiagabine has been shown to block the GABA-uptake mechanism in the brain (Fraser et al., 1999). Tiagabine revealed the presence of a GABA-uptake mechanism in the guinea pig myenteric plexus-longitudinal muscle. However, pretreatment with CP55,940 had no effect on the transient nature of the ethylenediamine-induced GABA inhibition, suggesting that the cannabinoid receptor stimulation has no effect on the GABA-uptake mechanism.

While low concentrations of anandamide acted in a similar manner to CP55,940, higher concentrations of anandamide *increased* the inhibition produced by ethylenediamine. Blockade of the cannabinoid $CB_1$-receptor mediated effects by the selective cannabinoid $CB_1$ receptor ligand, SR141716 increased the potentiating effect of anandamide on the ethylenediamine-evoked inhibition of electrically evoked contractions. This suggests that anandamide has a cannabinoid $CB_1$-receptor mediated inhibitory effect on the ethylenediamine-evoked release of GABA (blocked by SR141716) and at higher concentrations, a non-cannabinoid $CB_1$-receptor mediated potentiating effect on the ethylenediamine-induced inhibition of electrically evoked con- ractions. The selective cannabinoid $CB_2$ receptor ligand, SR144528 did not inhibit this effect, suggesting that this was not a cannabinoid $CB_2$-receptor mediated event either. Pretreatment with CGP54626A reduced this large inhibitory effect evoked by ethylenediamine by around 50%, showing that the other half of this response is neither cannabimetic nor GABAergic. The remaining inhibition produced by ethylenediamine was reduced by capsazepine, a vanilloid receptor antagonist, suggesting that the enhanced ethylenediamine inhibition is a result of anandamide activating the vanilloid VR1 receptor, as is seen in other preparations (Smart et al., 2000). This is in agreement with a recent study which also describes an increased release of GABA by anandamide in a hippocampal preparation that was due to vanilloid receptor activation (Davies and Al-Hayani, 2001). Another suggestion is that the ethylenediamine elicits another effect in addition to the release of GABA, which is enhanced by the presence of anandamide. At the highest concentration used, CGP54626A could not completely antagonize the effects of ethylenediamine, suggesting that the ethylenediamine does have some non-$GABA_B$-receptor-mediated effects. None of the concentrations of the anandamide used had any effect on the GABA-uptake mechanism, as the degree of reversal and the time taken for it to occur was unchanged. None of the concentrations of anandamide used in these experiments had any significant effect on the size of the electrically evoked contractions of the myenteric plexus-longitudinal muscle. Previously published work showed that the anandamide had an $EC_{50}$ of 8.82 μM in the myenteric plexus-longitudinal muscle (Pertwee et al., 1995). Given the fact that the $EC_{50}$ value for CP55,940 was higher in the present study than the previously published work, it may be that a higher concentration of anandamide would also be required to elicit an effect of comparable magnitude. A 10-fold higher concentration of anandamide did indeed produce a significant reduction in the size of the electrically evoked contractions.

In summary, the present study suggests that the activation of cannabinoid $CB_1$ and $GABA_B$ receptors in the myenteric plexus-longitudinal muscle of the guinea pig inhibits the electrically evoked cholinergic contractions through separate pathways. The endogenous cannabinoid receptor agonist, anandamide, inhibits the ethylenediamine-evoked release of GABA through a cannabinoid $CB_1$ receptor mediated event and potentiates the effect of ethylenediamine through a non-cannabinoid, non-GABA receptor mediated event, possibly through the activation of vanilloid VR1 receptors. Overall, the data raises the possibility that the cannabinoid receptor stimulation may play a role in the modulation of GABA function in the periphery.

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