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Modulation of the release of endogenous adenosine by cannabinoids in the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum

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1 Interactions between the cannabinoid system and the adenosine system were investigated in the myenteric plexus-longitudinal muscle (MPLM) of the guinea-pig ileum.

2 Electrically-evoked contractions of the MPLM were inhibited in a concentration dependent manner by exogenous adenosine and the adenosine receptor agonist 2-chloroadenosine. These inhibitory effects were reversed by the selective A_1 receptor antagonist DPCPX (20 nM).

3 Preincubation of the MPLM with the cannabinoid receptor agonist CP55,940 (1 nM) or the endogenous cannabinoid ligand anandamide caused a significant leftward shift in the concentration-effect curves to adenosine and 2-chloroadenosine.

4 Electrically-evoked contractions of the MPLM were inhibited in a concentration dependent manner by the adenosine uptake inhibitor dipyridamole. This inhibition was reversed by DPCPX (20 nM).

5 Pretreatment with CP55,940 (1 nM) or anandamide (10 μM) significantly reduced the inhibition produced by dipyridamole, an effect which was completely reversed by the selective CB_1 receptor ligand SR141716 (100 nM).

6 Electrically evoked adenosine release, measured in real time by means of adenosine-specific biosensors, was inhibited by CP55,940 (10 nM). This inhibition was blocked when CP55,940 was applied in the presence of SR141716 (100 nM).

7 These results confirm the presence of presynaptic CB_1 and A_1 receptors in the guinea-pig MPLM, and suggest that CB_1 receptor stimulation reduces electrically-evoked adenosine release. Overall the data raise the possibility that the cannabinoid system plays a role in the modulation of adenosine transmission in the MPLM.

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Abbreviations: DPCPX, 8-Cyclopentyl-1,3-dipropylxanthine; EHNA, *erythro*-9-(2-Hydroxy-3-nonyl)adenine; MPLM, myenteric plexus-longitudinal muscle preparation; NBTI, (S-(p-nitrobenzyl)-6-thio-inosine)

Introduction

There are a number of different presynaptic receptor types located on cholinergic neurones in the myenteric plexus-longitudinal muscle (MPLM) preparation of the guinea-pig ileum. They include the cannabinoid CB_1 and adenosine A_1 receptors. Activation of these receptors has been shown to inhibit electrically-evoked contractions of isolated strips of MPLM, through a reduction in acetylcholine release (Nitahara *et al*., 1995; Pertwee *et al*., 1996). Adenosine is released from the MPLM in response to depolarization by electrical stimulation (Alhumayyd & White, 1985) and is removed by uptake (Thorn & Jarvis, 1996) or broken down to inosine by adenosine deaminase (Bessodes *et al*., 1982). This released adenosine also activates A_1 receptors reducing the amount of acetylcholine release (Nitahara *et al*., 1995). Adenosine is recognized as a neuromodulator that suppresses transmitter release in both central (Dunwiddie & Fredholm, 1989) and peripheral (Christofi & Wood, 1993) neurones, and has been implicated in the control of reflex-evoked peristalsis (Poli & Pozzoli, 1997). Cannabinoid CB_1 receptor stimulation also modulates peristalsis (Heinemann *et al*., 1999), although the physiological role of cannabinoids in gut function is far from established.

There is growing evidence for interactions 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. CB_1 and A_1 receptors are co-localized on axons of cerebellar granule cells (Dar, 2000). Activation of these A_1 receptors modulates motor impairment induced by the major active constituent of cannabis Δ^9-THC, which is thought to occur through a post receptor effect on the cyclic AMP pathway (Dar, 2000).

The aim of the present study was to examine the effect of cannabinoid receptor stimulation on electrically evoked

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adenosine release and uptake in the MPLM of the guinea-pig ileum.

Methods

Preparation of the MPLM

Male Dunkin Hartley (contraction recordings) or Heston-2 (sensor recordings) guinea-pigs (450–550 g) were killed by cervical dislocation. The MPLM was dissected from the small intestine using the method described by Paton & 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.

Measurement of contraction

Strips of 4 cm of MPLM were mounted in 30 ml organ baths under an initial tension of 0.5 mN. 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, U.K.). Contractions of the MPLM were recorded by Dynamometer UF1 isometric transducers (Pioden Controls, U.K.), connected *via* a Conditioning Unit (Techman, U.K.) to a MX216 Chartrecorder (Electromed, U.K.).

Experimental design

The MPLM was electrically stimulated for the entire duration of the experiment, and it was noted that the size of contractions increased for up to 3 h, after which the size of contractions was stable. Hence, no drugs were added until 3 h had elapsed. Concentration-response curves to adenosine related compounds were performed cumulatively. Adenosine receptor antagonists were added 5 mins before the subsequent addition of compounds. Cannabinoid receptor agonists were added 20 mins before addition of any adenosine receptor agonists. In competition studies the CB_1 receptor ligand, SR141716 was added 30 mins before the addition of any subsequent 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. All experiments were performed with relevant parallel controls to ensure that no time dependent changes occurred.

Measurement of adenosine release

Direct measurement of adenosine release was achieved by using Pt microelectrode biosensors (*c.f.* Dale, 1998; Llaudet *et al*., 2002). The microelectrode sensors were fabricated from 50–250 μm diameter Pt wire and were a few mm in length. A three enzyme (adenosine deaminase, nucleoside phosphorylase and xanthine oxidase) cascade was entrapped in an electrodeposited matrix around the Pt microelectrode. This allows the amperometric detection of adenosine, as adenosine deaminase is specific for adenosine (Agarwal & Parks, 1978). The sensors are fast responding (10–90% rise time $\sim$2 s) and very sensitive (lower detection limit $\sim$10 nM) and allow real-time measurement of purine release. To determine whether the sensor specifically detected adenosine, we also used null sensors (possessing the polymeric matrix without enzymes). These were placed in the same position on the tissue as the adenosine sensors. In later experiments we made differential simultaneous recordings between the null and adenosine sensors. The null sensors failed to record any signal following stimulation of the ileum. By contrast the adenosine sensors recorded a transient signal. The sensors were thus responding to purine release. We did not ascertain that this was adenosine as opposed to inosine (an intermediate metabolite in the cascade and to which the sensor is sensitive). Nevertheless given the context of the work it is most likely that the sensor was responding to adenosine directly rather than its breakdown product inosine. The sensors were tested for their discriminative properties by comparing the responses to application of adenosine (10 μM) with responses to ATP, ADP, or AMP (10 μM each). The responses were proportional ($<$4% of response to equivalent dose of adenosine) to the free adenosine content produced by contamination/breakdown as measured by HPLC, demonstrating that the sensors possess a high degree of selectivity for adenosine.

To measure adenosine release, a segment of ileum was pinned flat with etched tungsten pins in a sylgard recording chamber that was continually superfused at a rate of around 6 ml min^{-1}. The sensing portion of the microelectrode was laid flat upon the tissue in gentle contact. A glass suction electrode, wrapped with Ag wire was used as a bipolar stimulating electrode. A train of 10–20 stimuli delivered through a stimulus isolator reliably evoked a widespread contraction of the tissue. The tissue was stimulated at regular intervals and the sensor responses recorded and acquired through an A/D converter to a PC for later analysis. The sensors were calibrated with adenosine prior to, during and after each experiment. In cases where gradual loss of sensor sensitivity was observed, the calibration closest in time to the measurements reported was used to convert the sensor current to adenosine concentration.

Drugs

CP55,940 ((−)-*cis*-3-[2- hydroxy-4-(1,1-dimethylheptyl) phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol) was a gift from Pfizer U.K., and anandamide ((all Z) -N- (2-hydroxyethyl)-5,8,11,14-eicosatetraenamide) from Tocris U.K. SR141716 (N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4,-di-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride) and SR144528 (N-[(1S)-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^{-1} in a soya oil/water (1 : 4) emulsion, shielded from light and kept at 4°C. EHNA (*erythro*-9-(2-hydroxy-3-nonyl)adenine) was obtained from Tocris, U.K., dissolved in distilled water and kept at 4°C. DPCPX (8-cyclopentyl-1,3-dipropylxanthine) dipyridamole

(2,6 - bis(diethanolamino) - 4,8 - dipiperindinopyrimido - [5,4-d] pyrimidine) and NBTI (S-(p-nitrobenzyl)-6-thio-inosine) were obtained from Sigma, U.K., dissolved in ethanol and kept at −20°C. Adenosine (9-β-D-ribofuranosyladenine) and 2-chloroadenosine (6-amino-2-chloropurine riboside) were obtained from Sigma U.K., dissolved in distilled water, and kept at 4°C.

Analysis of data

Each value is expressed as the mean ± standard error of mean of experiments on tissues obtained from at least six individual animals. The effects of cannabinoid and adenosine receptor agonists are expressed as 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 with 95% confidence limits, using GraphPAD Prism statistical software (GraphPAD Software, CA., U.S.A.). 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 ANOVA test was performed, followed by a *post-hoc* Dunnett's test to calculate significant differences between multiple test groups.

Results

Effect of adenosine A_1 and cannabinoid CB_1 receptor stimulation on the electrically evoked contractions of the MPLM

Electrically evoked contractions of the MPLM were inhibited by the adenosine receptor agonist 2-chloroadenosine and exogenous adenosine in a concentration dependent manner ($pEC_{50} = 5.52$, 95% confidence limits of 5.44 and 5.68 for 2-chloroadenosine and $pEC_{50} = 5.49$, 95% confidence limits of 5.42 and 5.52 for adenosine, $n = 6$, Figure 1). These effects were reversed by the selective A_1 receptor antagonist DPCPX ((Bruns *et al*., 1987), 20 nM, $n = 6$, data not shown). Addition of DCPCX (20 nM, $n = 6$) alone significantly increased the size of the electrically evoked contractions by 18 ± 3%.

Preincubation of the MPLM with the cannabinoid receptor agonist CP55,940 (20 min, 1 nM, $n = 6$) caused significant ($P < 0.05$) leftward shifts in the concentration-effect curves to both adenosine and 2-chloroadenosine. The pEC_{50} for adenosine was increased to 5.98 (95% confidence limits of 5.70 and 6.25, Figure 1a) and 2-chloroadenosine to 5.95 (95% confidence limits of 5.68 and 6.14, Figure 1c). The endogenous cannabinoid receptor agonist anandamide (20 min, 10 μM, $n = 6$) also caused significant ($P < 0.05$) leftward shifts in the concentration effect curves to both adenosine and 2-chloroadenosine. The pEC_{50} for adenosine was increased to 6.01 (95% confidence limits of 5.78 and 6.39, Figure 1b) and 2-chloroadenosine to 5.98 (95% confidence limits of 5.95 and 6.29, Figure 1d). The concentrations of CP55,940 and anandamide used produced a small reduction of the magnitude of the electrically-evoked contractions (11 ± 5% and 9 ± 4%, respectively).

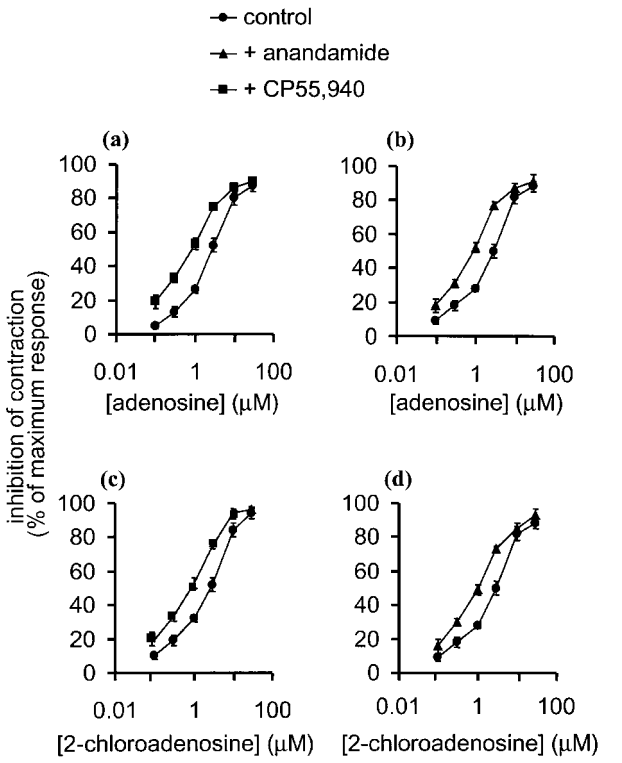

Figure 1 Effect of cannabinoid receptor agonists, CP55,940 (1 nM; a and c) and anandamide (10 μM; b and d) on mean concentration-response curves to adenosine (a and b) and the selective adenosine A_1 receptor agonist, 2-chloroadenosine (c and d). All data are expressed as means ± s.e.means with $n = 6$ in each case.

Pretreatment of the MPLM with the selective CB_1 receptor ligand SR141716 (Rinaldicarmona *et al*., 1994) had no effect on the concentration effect curves to 2-chloroadenosine ($n = 6$). SR141716 alone caused a small increase in the magnitude of the electrically evoked contraction of 17 ± 10% ($n = 6$).

Effect of EHNA on electrically evoked contractions of the MPLM

Electrically evoked contractions of the MPLM were slightly reduced (15 ± 8%) by the inhibitor of adenosine deaminase, EHNA (Bessodes *et al*., 1982; 1 to 300 μM, $n = 6$). A sample chart recorder trace of the effect of EHNA is shown in Figure 2d. Addition of DPCPX (20 nM) reversed the inhibitory effects of EHNA.

Effect of dipyridamole on electrically evoked contractions of the MPLM

Electrically evoked contractions of the MPLM were inhibited by the addition of the adenosine uptake inhibitor dipyridamole (Meester *et al*., 1998) in a concentration dependent manner ($pEC_{50} = 6.53$, 95% confidence limits of 6.64 and 6.49, $n = 6$, Figure 3). A typical chart recorder trace illustrating the

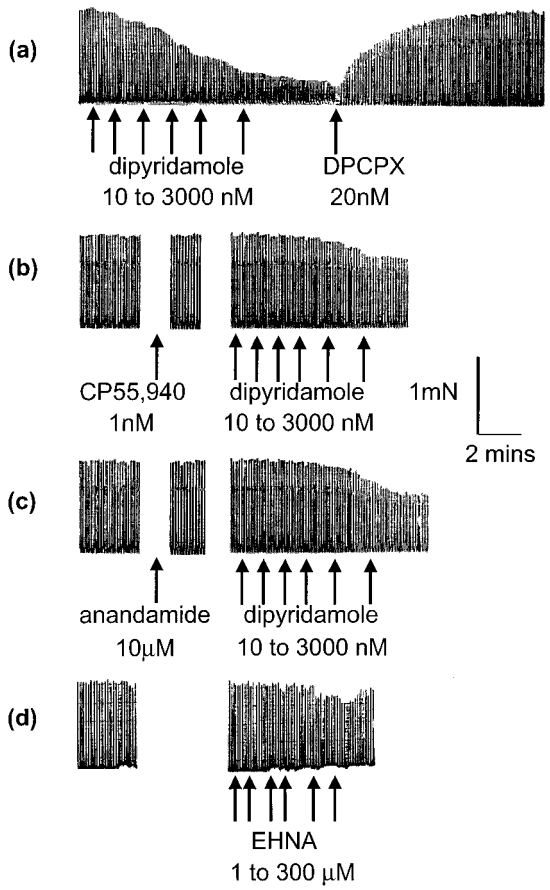

Figure 2 Sample chart recorder traces of the effects of the adenosine uptake inhibitor dipyridamole (10 to 3000 nM) and the adenosine amidase inhibitor EHNA (1 to 300 μM) on the electrically evoked contractions of the guinea-pig MPLM. (a) The inhibitory effect of cumulative concentrations of dipyridamole and its reversal by the selective adenosine A_1 receptor antagonist, DPCPX (20 nM). Arrows indicate the point at which compounds were administered, upward deflection denotes an electrically evoked contraction. (b) The effect of pretreatment with CP55,940 (1 nM, 20 mins) on the inhibitory effects of dipyridamole. The initial part of trace denotes the magnitude of contractions before the addition of CP55,940, the second part denotes the effect of CP55,940 prior to dipyridamole (c) the effect of pretreatment with anandamide (10 μM, 20 mins) on the inhibitory effects of dipyridamole. The initial part of trace denotes the magnitude of contractions before the addition of anandamide, the second part denotes the effect of anandamide prior to dipyridamole. (d) The inhibitory effects of EHNA.

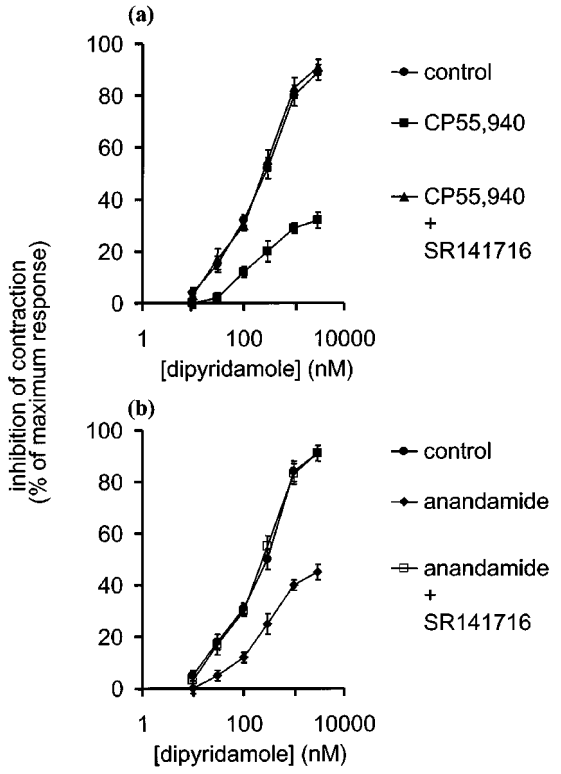

Figure 3 The effect of the cannabinoid receptor agonists CP55,940 (1 nM; a) and anandamide (10 μM; b) on the inhibition of electrically evoked contractions of guinea-pig MPLM by the adenosine uptake inhibitor dipyridamole (10 to 3000 nM), and the blockade of the effects of the cannabinoid receptor agonists by the selective CB_1 receptor ligand SR141716 (100 nM; a and b). All data are expressed as means$\pm$s.e.means with $n=6$ in each case.

inhibition produced by dipyridamole is shown in Figure 2a. The inhibitory effect of dipyridamole could not be reversed by washing with Krebs, so only one concentration effect curve was performed on a single MPLM preparation. The inhibitory effect could be reversed by the selective A_1 receptor antagonist DPCPX (20 nM, $n=6$, Figure 2a).

Effect of CB_1 receptor stimulation on the inhibition of electrically evoked contractions by dipyridamole

Pretreatment of the MPLM with CP55,940 (20 min, 1 nM, $n=6$) significantly reduced the maximum inhibition produced by dipyridamole from $92\pm16\%$ to $34\pm2\%$ ($P<0.01$, Figure 3a). A typical chart recorder trace of this experiment is shown in Figure 2b. The reduction in dipyridamole inhibition by CP55,940 was completely reversed by the selective CB_1 receptor ligand SR141716 (100 nM, $n=6$, Figure 3a).

Pretreatment of the MPLM with anandamide (20 mins, 10 μM, $n=6$) also significantly reduced the maximum inhibition produced by dipyridamole from $92\pm16\%$ to $49\pm3\%$ ($P<0.05$, Figure 3b). A typical chart recorder trace of this experiment is shown in Figure 2c. The reduction in dipyridamole inhibition by anandamide was also completely reversed by SR141716 (100 nM, $n=6$, Figure 3b).

Effect of CB_1 receptor stimulation on the inhibition of electrically evoked contractions by NBTI

Electrically evoked contractions of the MPLM were inhibited by $53\pm5\%$ by the addition of another adenosine uptake inhibitor, NBTI ((Lloyd & Fredholm, 1995), 300 nM, $n=6$, Figure 4). Pretreatment of the MPLM with CP55,940 (20 mins, 1 nM, $n=6$) also significantly reduced the maximum inhibition produced by NBTI to $10\pm3\%$ ($P<0.01$, Figure 4). For comparison, the highest concentration of dipyridamole (3000 nM) and the effect on this by CP55,940 (1 nM) are also shown in Figure 4.

Direct measurement of electrically evoked adenosine release

Focal stimulation evoked a transient rise in adenosine concentration at the surface of the tissue, with a maximum

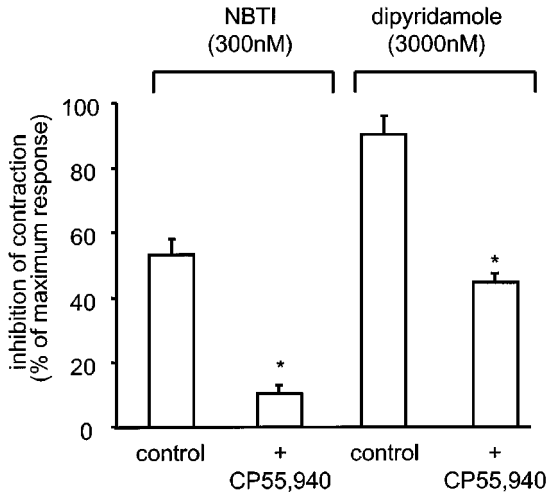

Figure 4 The effect of the cannabinoid receptor agonist CP55,940 (1 nM) on the inhibition of electrically evoked contractions of guinea-pig MPLM by dipyridamole (3000 nM) and NBTI (300 nM). All data are expressed as means ± s.e.means with $n=6$ in each case. *Indicates a significant difference from control, $P<0.01$.

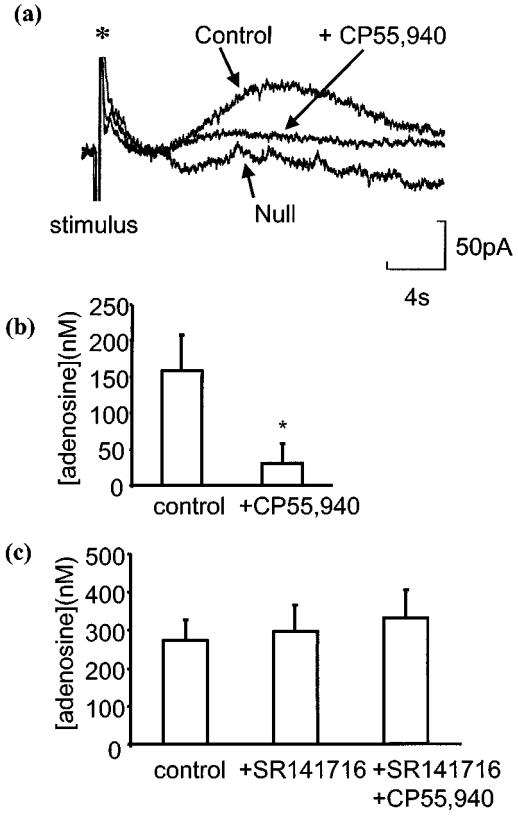

Figure 5 The effect of 10 nM CP55,940 on adenosine release in the MPLM as recorded by adenosine sensors on the surface of the tissue. (a) Example traces of null and adenosine sensor recordings in the absence and presence of CP55,940. The calibration bar of 50 pA is equivalent to a change in adenosine concentration of 80 nM. (b) Inhibition of peak evoked adenosine increase by CP55,940. (c) Prevention by SR141716 of inhibition by CP55,940. All data are expressed as means ± s.e.means with $n=4$ for the CP55,940 data and $n=3$ for the SR141716 data. *Indicates a significant difference from control, $P<0.01$.

of 207 ± 41 nM ($n=7$) occurring 4.0 ± 1.3 s after application of the stimulus. In four cases CP55,940 (10 nM) was applied and this inhibited the peak adenosine transient by 81% (control and CP55,940 respectively 158 ± 50 nM and 31 ± 28 nM, $n=4$, $P=0.014$, paired sample t-test, Figure 5a,b). In a further three cases we tested whether the inhibition of adenosine release by CP55,940 was prevented by pretreatment with the CB_1 antagonist SR141716 at 100 nM. In the control the peak adenosine release was 272 ± 55 nM. In the presence of SR141716 and CP55,940 the peak adenosine release was statistically unchanged at 333 ± 74 nM ($n=3$, Figure 5c). We therefore conclude that SR14176 blocked the action of the CB agonist and that the actions of CP55,940 on adenosine release were mediated by a specific receptor.

Discussion

Our results confirm the presence of adenosine A_1 receptors in the MPLM preparation of the guinea-pig ileum. Activation of A_1 receptors reduced the electrically-evoked contractions of MPLM, which has been shown previously to be due to a reduction in acetylcholine release (Nitahara *et al*., 1995). It is unlikely that these receptors are located post-synaptically, because unlike in the rat (Nicholls & Hourani, 1997), in the guinea-pig ileum exogenous adenosine does not inhibit cholinergically evoked contractions (Lee, 1998).

Recent studies have demonstrated interactions between cannabinoid and other receptor systems (Smart *et al*., 2000), which prompted the investigation into concurrent stimulation of CB_1 and A_1 receptors. Cannabinoid CB_1 receptors have been thoroughly described in the MPLM, where their activation inhibits the electrically-evoked contractions, also by a presynaptic inhibition of acetylcholine release (Coutts & Pertwee, 1997). We have shown previously that CB_1 receptor mediated inhibition of transmitter release in this tissue extends to GABA (Begg *et al*., 2001). Pretreatment with the cannabinoid receptor agonists CP55,940 or anandamide caused significant leftward shifts in the concentration effect curves to both adenosine and 2-chloroadenosine. This combined inhibitory effect of CB_1 and A_1 receptor stimulation may arise as CB_1 receptors are believed to inhibit the release of acetylcholine by direct effects on Ca^{2+} and K^+ channels (Mackie *et al*., 1995), whereas A_1 receptors are thought to influence acetylcholine release by affecting cyclic AMP levels (Dunwiddie & Fredholm, 1989).

The concentrations of CP55,940 and anandamide used only slightly reduced the magnitude of the electrically evoked contractions. A previous study suggested the concentration of anandamide used, even in the absence of an inhibitor of anandamide metabolism such as PMSF, should have produced a large inhibitory effect on the electrically evoked contractions of the MPLM (Pertwee *et al*., 1995). No clear reason for this discrepancy can be offered although in the present study only a single concentration of anandamide was used whereas in the published study a cumulative concentration-response curve was established. In addition, the vehicles used for anandamide were different in the two studies.

An inhibitor of adenosine uptake, dipyridamole (Meester *et al*., 1998) concentration dependently inhibited the electrically-

evoked contractions of the MPLM. This inhibitory effect was reversed with DPCPX, suggesting that the inhibition of contractions were a result of A_1 receptor activation. Adenosine is released upon electrical stimulation (Alhumayyd & White, 1985), and inhibition of adenosine uptake leads to an increased concentration of extracellular adenosine, which activates presynaptic A_1 receptors, reducing electrically-evoked contractions. Our results confirm the electrically-evoked release of adenosine, and that uptake is the major route of its removal. In contrast, an inhibitor of adenosine metabolism EHNA had little effect on the electrically-evoked contractions of the MPLM, suggesting that metabolism is not a major pathway for the removal of electrically-evoked released adenosine in this preparation.

Cannabinoid receptor stimulation significantly reduced the inhibitory effects of dipyridamole. Concentrations of either CP55,940 or anandamide that were not sufficient to significantly alter the magnitude of the electrically-evoked contractions were able to markedly reduce the inhibitory effects of dipyridamole. SR141716 completely reversed this effect showing that it was mediated through CB_1 receptors. This suggests that in addition to being able to reduce acetylcholine release, activation of presynaptic CB_1 receptors also reduces the electrically-evoked release of adenosine. As with all neurotransmitters, the electrically-evoked release of adenosine is a Ca^{2+}-dependent event (Lloyd *et al*., 1993). The reduction in Ca^{2+} channel opening following CB_1 receptor stimulation (Mackie & Hille, 1992) maybe responsible for the reduction in adenosine release following pretreatment with CP55,940 or anandamide. Cannabinoid receptor stimulation also reduced the inhibition of electrically-evoked contractions by another adenosine uptake inhibitor, NBTI, confirming the results observed with dipyridamole.

The selective A_1 receptor antagonist DPCPX when applied alone increased the magnitude of the electrically-evoked contractions, again suggesting that adenosine is released in response to electrical stimulation. The selective CB_1 receptor ligand SR141716 also increased the magnitude of the electrically-evoked contractions. This may suggest a tonic release of endogenous cannabinoids, but since SR141716 had no effect on the inhibition produced by 2-chloroadenosine this may not be the case. The observed increase in electrically-evoked contractions may instead be due to the inverse-agonist effect of SR141716 (Landsman *et al*., 1997).

An independent and direct way of recording adenosine release was applied to further test the possibility that adenosine release is modulated by CB receptor stimulation. Using an adenosine sensor (Llaudet *et al*., 2002), we observed that electrically evoked adenosine release was inhibited by CP55,940 but not when the tissue was pre-incubated with the CB_1 antagonist SR141716. These findings are consistent with the pharmacological results and confirm that CB_1 receptor stimulation inhibits adenosine release in the MPLM.

In summary, these results confirm the presence of presynaptic CB_1 and A_1 receptors in the guinea-pig MPLM. The inhibitory effect of adenosine uptake blockers was reduced by CP55,940 and anandamide suggesting that CB_1 receptor stimulation reduces electrically-evoked adenosine release. This was supported by direct measurement of adenosine release. Overall the data add to the body of evidence suggesting that the cannabinoids system plays a wide role in the modulation of neurotransmission in the MPLM.

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References

AGARWAL, R.P. & PARKS, R.E. (1978). Adenosine deaminase from human erythrocytes. *Methods Enzymol.,* **51,** 502 – 507.

ALHUMAYYD, M. & WHITE, T.D. (1985). Adrenergic and possible nonadrenergic sources of adenosine 5′-triphosphate release from nerve varicosities isolated from ileal myenteric plexus. *J. Pharm. Exp. Ther.,* **233,** 796 – 800.

BEGG, M., MOLLEMAN, A. & PARSONS, M.E. (2001). Modulation of the release of endogenous GABA by cannabinoids in the myenteric plexus longitudinal muscle preparation of the guinea-pig ileum. *Eur. J. Pharmacol.,* **434,** 87 – 94.

BESSODES, M., BASTIAN, G., ABUSHANAB, E., PANZICA, R.P., BERMAN, S.F., MARCACCIO, E.J., CHEN, S.F., STOECKLER, J.D. & PARKS, R.E. (1982). Effect of chirality in *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA) on adenosine-deaminase inhibition. *Biochem. Pharm.,* **31,** 879 – 882.

BRUNS, R.F., FERGUS, J.H., BADGER, E.W., BRISTOL, J.A., SANTAY, L.A., HARTMAN, J.D., HAYS, S.J. & HUANG, C.C. (1987). Binding of the A_1-selective adenosine antagonist 8-cyclopentyl- 1,3-dipropylxanthine to rat-brain membranes. *N.S. Arch. Pharm.,* **335,** 59 – 63.

CHRISTOFI, F.L. & WOOD, J.D. (1993). Presynaptic inhibition by adenosine-A_1-receptors on guinea-pig small intestinal myenteric neurons. *Gastroenterology,* **104,** 1420 – 1429.

COLQUHOUN, D. (1971). *Lectures on Biostatistics*. Oxford: Oxford University Press.

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.

DALE, N. (1998). Delayed production of adenosine underlies temporal modulation of swimming in frog embryo. *J. Physiol.,* **511,** 265 – 272.

DAR, M.S. (2000). Cerebellar CB_1 receptor mediation of Δ^9-THC-induced motor incoordination and its potentiation by ethanol and modulation by the cerebellar adenosinergic A_1 receptor in the mouse. *Brain Res.,* **864,** 186 – 194.

DUNWIDDIE, T.V. & FREDHOLM, B.B. (1989). Adenosine A_1 receptors inhibit adenylate-cyclase activity and neurotransmitter release and hyperpolarize pyramidal neurons in rat hippocampus. *J. Pharm. Exp. Ther.,* **249,** 31 – 37.

HEINEMANN, A., SHAHBAZIAN, A. & HOLZER, P. (1999). Cannabinoid inhibition of guinea-pig intestinal peristalsis via inhibition of excitatory and activation of inhibitory neural pathways. *Neuropharmacology,* **38,** 1289 – 1297.

IRVING, A.J., COUTTS, A.A., HARVEY, J., RAE, M.G., MACKIE, K., BEWICK, G.S. & PERTWEE, R.G. (2000). Functional expression of cell surface cannabinoid CB_1 receptors on presynaptic inhibitory terminals in cultured rat hippocampal neurons. *Neuroscience,* **98,** 253 – 262.

LANDSMAN, R.S., BURKEY, T.H., CONSROE, P., ROESKE, W.R. & YAMAMURA, H.I. (1997). SR141716A is an inverse agonist at the human cannabinoid CB_1 receptor. *Eur. J. Pharmacol.,* **334,** R1 – R2.

LEE, J.J. (1998). Presynaptic receptors in the guinea-pig ileum: Signaling pathways and interactions. PhD Thesis, University of Hertfordshire, Hatfield.

LLAUDET, E., BOTTING, N.P., CRAYSTON, J.A. & DALE, N. (2002). A 3 enzyme sensor for detecting purines from CNS. *Biosens. Bioelectronics* (In Press).

LLOYD, H.G.E. & FREDHOLM, B.B. (1995). Involvement of adenosine-deaminase and adenosine kinase in regulating extracellular adenosine concentration in rat hippocampal slices. *Neurochem. Intern.,* **26,** 387 – 395.

LLOYD, H.G.E., LINDSTROM, K. & FREDHOLM, B.B. (1993). Intracellular formation and release of adenosine from rat hippocampal slices evoked by electrical-stimulation or energy depletion. *Neurochem. Intern.,* **23,** 173 – 185.

MACKIE, K. & HILLE, B. (1992). Cannabinoids inhibit N-type calcium channels in neuroblastoma glioma-cells. *Proc. Nat. Acad. Sci. U.S.A.,* **89,** 3825 – 3829.

MACKIE, K., LAI, Y., WESTENBROEK, R. & MITCHELL, R. (1995). Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in ATT20 cells transfected with rat-brain cannabinoid receptor. *J. Neurosci.,* **15,** 6552 – 6561.

MEESTER, B.J., SHANKLEY, N.P., WELSH, N.J., MEIJLER, F.L. & BLACK, J.W. (1998). Pharmacological analysis of the activity of the adenosine uptake inhibitor, dipyridamole, on the sinoatrial and atrioventricular nodes of the guinea-pig. *Br. J. Pharmacol.,* **124,** 729 – 741.

NICHOLLS, K. & HOURANI, S.M.O. (1997). Characterization of adenosine receptors on rat ileum, ileal longitudinal muscle and muscularis mucosae. *Eur. J. Pharmacol.,* **338,** 143 – 150.

NITAHARA, K., KITTEL, A., LIANG, S.D. & VIZI, E.S. (1995). A_1-receptor-mediated effect of adenosine on the release of acetylcholine from the myenteric plexus – role and localization of ecto-atpase and 5′-nucleotidase. *Neuroscience,* **67,** 159 – 168.

PATON, W.D.M. & ZAR, M.A. (1968). The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle fibres. *J. Physiol.,* **194,** 13 – 33.

PERTWEE, R.G., FERNANDO, S.R., GRIFFIN, G., ABADJI, V. & MAKRIYANNIS, A. (1995). Effect of phenylmethylsulfonyl 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., NASH, J.E. & COUTTS, A.A. (1996). Further evidence for the presence of cannabinoid CB_1 receptors in guinea-pig small intestine. *Br. J. Pharmacol.,* **118,** 2199 – 2205.

POLI, E. & POZZOLI, C. (1997). Histamine H_3 receptors do not modulate reflex-evoked peristaltic motility in the isolated guinea-pig ileum. *Eur. J. Pharmacol.,* **327,** 49 – 56.

RINALDICARMONA, 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. & LEFUR, G. (1994). SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS. Lett.,* **350,** 240 – 244.

SMART, D., GUNTHORPE, M.J., JERMAN, J.C., NASIR, S., GRAY, J., MUIR, A.I., CHAMBERS, J.K., RANDALL, A.D. & DAVIS, J.B. (2000). The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR_1). *Br. J. Pharmacol.,* **129,** 227 – 230.

THORN, J.A. & JARVIS, S.M. (1996). Adenosine transporters. *General Pharmacology,* **27,** 613 – 620.

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