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Vasodilator actions of abnormal-cannabidiol in rat isolated small mesenteric artery

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1 The nonpsychoactive cannabinoid abnormal-cannabidiol (*trans*-4-[3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol) (abn-cbd) produced concentration-dependent relaxation of methoxamine-precontracted rat small mesenteric artery. Endothelial removal reduced abn-cbd potency six-fold without affecting the maximum relaxation.

2 In endothelium-intact vessels, abn-cbd was less potent under 60 mM KCl-induced tone and inhibited by combination of L-N^G-nitroarginine methyl ester (L-NAME) (nitric oxide synthase inhibitor; 300 μM), apamin (small conductance Ca^{2+}-activated K^+ channels inhibitor; 50 nM) and charybdotoxin (inhibitor of intermediate conductance Ca^{2+}-activated K^+ channels and large conductance Ca^{2+}-activated K^+ channels BK_{Ca}; 50 nM). L-NAME alone or in combination with either toxin alone had little effect.

3 In intact vessels, relaxations to abn-cbd were inhibited by SR 141716A (cannabinoid receptor antagonist; 1 or 3 μM). Concomitant addition of L-NAME, apamin and charybdotoxin had no further effect. Other cannabinoid receptor antagonists either had little (SR 144528; 1 μM and AM 251; 1 μM) or no effect (AM 630; 10 μM and AM 281; 1 μM). Inhibition of gap junctions, $G_{i/o}$ protein coupling and protein kinase A also had no effect.

4 Endothelium-independent relaxation to abn-cbd was unaffected by L-NAME, apamin plus charybdotoxin or capsaicin (10 μM). Abn-cbd inhibited $CaCl_2$-induced contractions in vessels with depleted intracellular Ca^{2+} stores and stimulated with methoxamine or KCl. This was insensitive to SR 141716A (3 μM) but greatly reduced in vessels stimulated with ionomycin (Ca^{2+} ionophore; 1 μM).

5 We conclude that abn-cbd relaxes the rat small mesenteric artery by endothelium-dependent activation of K^+ channels via SR 141716A-sensitive pathways, which do not involve CB_1 and CB_2 receptors. It also causes endothelium-independent, SR 141716A-insensitive, relaxation by inhibiting Ca^{2+} entry through voltage-gated Ca^{2+} channels.
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Abbreviations: Abn-cbd, abnormal-cannabidiol (*trans*-4-[3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol); BK_{Ca}, large conductance Ca^{2+}-activated K^+ channels; E_{max}, maximum effect; IK_{Ca}, intermediate conductance Ca^{2+}-activated K^+ channels; L-NAME, L-N^G-nitroarginine methyl ester; O-1602, (*trans*-4-[3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-methyl-1,3-benzenediol); pEC_{50}, negative logarithm of the concentration causing 50% of the maximum response; Rp-cAMPS, Rp-adenosine 3′,5′-cyclic monophosphothioate triethylamine; SK_{Ca}, small conductance Ca^{2+}-activated K^+ channels; VGCC, voltage-gated Ca^{2+} channels

Introduction

Cannabinoids such as Δ^9-tetrahydrocannabinol, the psychoactive principle in marijuana, and the putative endocannabinoid anandamide induce vasorelaxation in addition to their neurobehavioural effects (see Hillard, 2000 for review). Thus, they might provide future treatment for cardiovascular disorders, especially if their vascular actions could be dissociated from psychoactive effects. Most of the central and peripheral actions of cannabinoids have been shown to be mediated by cannabinoid receptors, of which CB_1, CB_{1A} and CB_2 subtypes have been cloned (see Howlett *et al*., 2002 for review). CB_1 and CB_{1A} receptors are generally considered to be central receptors (Matsuda *et al*., 1990; Shire *et al*., 1995), whereas CB_2 receptors occur mainly in immune cells (Munro *et al*., 1993; Facci *et al*., 1995). These receptors are coupled to $G_{i/o}$ proteins and inhibit adenylyl cyclase, but only CB_1 receptors have been shown to inhibit N-, and P/Q-type Ca^{2+} channels and stimulate opening of inwardly rectifying and A-type K^+ channels (see Felder & Glass, 1998 for review). Some time ago, it was shown that prolonged use of cannabis in man produces a long-lasting decrease in both blood pressure and heart rate (Benowitz & Jones, 1974). More recently, cannabinoids were found to cause prolonged hypotension in anaesthetised animals apparently via activation of CB_1 receptors (Varga *et al*., 1995; Lake *et al*., 1997). CB_1 receptors have also been identified in cat cerebrovascular smooth muscle, where their activation causes vasorelaxation linked to activation of $G_{i/o}$ proteins and inhibition of L-type Ca^{2+} channels (Gebremedhin *et al*., 1999). In rat renal arterioles, anandamide appears to produce nitric oxide-dependent relaxation by

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activation of endothelial CB_1 receptors (Deutsch *et al*., 1997). However, other CB_1 receptor-independent pathways are also well documented in different isolated blood vessels. These include metabolism of anandamide into arachidonic acid, and thence to other vasodilator eicosanoids (Ellis *et al*., 1995; Pratt *et al*., 1998), as well as activation of vanilloid (VR1) receptors located on perivascular sensory nerves (Zygmunt *et al*., 1999; Ralevic *et al*., 2000). A CB_2 receptor agonist has also been shown to reduce blood pressure without behavioural activity *in vivo* (Hanus *et al*., 1999), but the presence of the CB_2 receptor in vascular tissues remains to be demonstrated.

Abnormal-cannabidiol (abn-cbd; *trans*-4-[3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol), a synthetic analogue of the plant-derived cannabinoid cannabidiol, was reported to produce profound hypotension in dogs without behavioural effects (Adams *et al*., 1977). Its parent compound, cannabidiol, is itself inactive in assays for both neurobehavioural (Mansbach *et al*., 1996) and cardiovascular effects (Adams *et al*., 1977), and shows low affinity for cannabinoid CB_1 and CB_2 receptors (Munro *et al*., 1993; Showalter *et al*., 1996). Járai *et al*. (1999), who followed up these interesting observations, demonstrated that abn-cbd caused hypotension and relaxation in the perfused mesenteric bed of both CB_1 and CB_2 receptor knockout mice. In rat perfused mesenteric bed, relaxation to abn-cbd, like that to anandamide, was found to be endothelium-dependent and sensitive to the commonly used CB_1 receptor antagonist SR 141716A at micromolar concentrations. O-1602 (*trans*-4-[3-methyl-6-(1-methylethenyl)-2-cyclohexen - 1 - yl] - 5-methyl-1,3-benzenediol), an analogue of abn-cbd in which the pentyl side chain was shortened to a methyl group, also showed similar vasodilator activity. It was proposed that abn-cbd and O-1602 are agonists of a putative new receptor expressed in the endothelium of rat mesenteric bed. This endothelial receptor, distinct from the currently known cannabinoid receptors, has also been suggested to mediate anandamide-induced relaxation in the whole mesenteric bed of the rat and has been referred to as a putative 'endothelial anandamide receptor' (Wagner *et al*., 1999; Járai *et al*., 1999). As the other putative endocannabinoid 2-arachidonyl glycerol and some synthetic cannabinoids (WIN 55, 212-2, HU210 and CP 55, 940) cause either endothelium-independent or little relaxation in rat mesenteric artery (White & Hiley, 1998b; Wagner *et al*., 1999), anandamide, abn-cbd and O-1602 are the only agonists currently proposed for this putative endothelial receptor. Anandamide is also known to produce endothelium-independent relaxation by activating vanilloid receptors and thereby stimulating CGRP release from perivascular sensory nerves in rat mesenteric arteries (Zygmunt *et al*., 1999; Ralevic *et al*., 2000; White *et al*., 2001), so activation of this endothelial receptor might serve as a parallel pathway for anandamide-induced relaxations in this vascular bed. In fact, this receptor might mediate mesenteric vasorelaxation caused by elevated levels of anandamide during endotoxic shock (Wagner *et al*., 1999). However, the mechanisms by which activation of this endothelial receptor cause vasorelaxation remain to be clarified.

In this study, we aimed to investigate further the mechanisms underlying relaxation induced by abn-cbd in rat isolated small mesenteric artery. The involvement of endothelium-derived factors, K^+ and Ca^{2+} channels, gap junctions, $G_{i/o}$ proteins, and vanilloid receptor activation in relaxation to abn-cbd was examined in the myograph-mounted preparation. The effects of a range of cannabinoid CB_1 and CB_2 receptor antagonists, including SR 141716A and SR 144528, on concentration – response curves of abn-cbd were also assessed.

Methods

Myograph studies

Male Wistar rats (300 – 400 g; Tucks, Rayleigh, Essex or Charles River U.K. Ltd, Kent) were killed with an overdose of sodium pentobarbitone (120 mg kg^{-1}, i.p., Sagatal, Rhone Mérieux, Harlow, Essex). Third-order branches of superior mesenteric artery (250 – 400 μm diameter) were removed and segments (2 mm) were mounted in a Mulvany-Halpern myograph (Model 500A, J.P. Trading, Aarhus, Denmark). Vessels were normalised as described previously (White & Hiley, 1997) in gassed (95% O_2/5% CO_2) Krebs – Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, D-glucose 5.6; except where stated, all experiments were carried out in the presence of indomethacin (10 μM). In all vessels, the integrity of the endothelium was assessed by precontracting the vessel with methoxamine (an α_1-adrenoceptor agonist; 10 μM) followed by relaxation with carbachol (10 μM); relaxations greater than 90% were designated as endothelium-intact. When endothelium was not required, it was removed by rubbing the intima with a human hair; carbachol-induced relaxation of less than 10% indicated successful removal. Removal of the endothelium had no significant effect on methoxamine-induced tone in the same preparations (with endothelium 14.0 ± 0.9 mN; after endothelial removal 14.2 ± 0.9 mN; $n = 52$).

Experiments in the presence of extracellular Ca^{2+}

After the test for endothelial integrity, vessels were left for 30 min. Vessels were then precontracted submaximally with methoxamine (10 μM), and this was followed by construction of a cumulative concentration – response curve to abn-cbd.

To investigate relaxation mechanisms, cannabinoid antagonists (SR 141716A, AM 251, AM 281, SR 144528 or AM 630) or other agents (capsaicin, 18α-glycyrrhetinic acid or Rp-adenosine 3′,5′-cyclic monophosphothioate triethylamine (Rp-cAMPS)) were added to the myograph bath 30 min before, and were present during, construction of the concentration – response curve. In the case of pertussis toxin, preincubation was for 2 h. In experiments investigating the effects of abn-cbd on voltage-gated Ca^{2+} channels (VGCC), vessels were precontracted with high K^+ (60 mM) Krebs – Henseleit solution, which was prepared by equimolar substitution of NaCl for KCl in the standard Krebs – Henseleit buffer described previously. The mean tension generated by 60 mM KCl (13.6 ± 1.6 mN) was similar to the tone induced in the same vessels by 10 μM methoxamine in the test for endothelium (13.6 ± 1.4 mN; $n = 14$).

The actions of abn-cbd were also examined in the presence of the nitric oxide synthase inhibitor, L-N^G-nitroarginine methyl ester (L-NAME) (300 μM), alone or in combination with K^+ channel blockers (apamin and charybdotoxin, or

iberiotoxin; all at 50 nM). All these were added 30 min before, and were then present throughout, the construction of the concentration – response curve. Although application of K^+ channel blockers and L-NAME rarely increased the resting tension, the vasoconstrictor effect of methoxamine was greatly potentiated. Therefore, a reduced concentration (1 – 3 μM) of methoxamine was used to precontract vessels in order to obtain an equivalent level of tone to that evoked in the absence of these inhibitors. In these preparations, the mean contraction to methoxamine in the test for endothelium was 17.2 ± 1.1 mN, as compared with 17.5 ± 1.5 mN ($n = 37$) in the presence of L-NAME (alone or with K^+ channel blockers).

As washing could not reverse the effects of abn-cbd, only a single concentration – response curve was constructed in each preparation. A vehicle (ethanol) control for the abn-cbd was obtained by adding appropriate volume of vehicle to methoxamine-precontracted vessels. Since the cannabinoid antagonists, capsaicin and 18α-glycyrrhetinic acid were dissolved in either ethanol or dimethyl sulphoxide, the effects of either vehicle alone on relaxation to abn-cbd were also examined.

Ca^{2+}-free experiments

Influx of extracellular Ca^{2+} through plasma membrane Ca^{2+} channels was examined in endothelium-denuded mesenteric arteries depleted of intracellular Ca^{2+} stores according to the methods described previously (White & Hiley, 1998c). Briefly, extracellular Ca^{2+} was removed by washing vessels with Ca^{2+}-free Krebs – Henseleit solution (composition the same as normal Krebs – Henseleit buffer but with $CaCl_2$ omitted). EGTA (1 mM) was added to the solution in the organ bath, followed by a series of additions of 10 μM methoxamine in order to deplete intracellular Ca^{2+} stores as shown by loss of the contractile response. Vessels were then washed with Ca^{2+}-free Krebs – Henseleit solution, 10 μM methoxamine was added, and a cumulative concentration – response curve to $CaCl_2$ (10 μM – 10 mM) was then obtained. As concentration – response curves to $CaCl_2$ were found to be reproducible in any given vessel, after a control curve and the washing process, a second test curve was constructed in the presence of abn-cbd (with 30 min preincubation). Contractions were expressed as a percentage of the maximum contraction induced by $CaCl_2$ in the vessel in the presence of 10 μM methoxamine alone. The above experiments were repeated with methoxamine (10 μM) being substituted by KCl (60 mM) and the percentage of the maximum $CaCl_2$-induced contraction was similarly calculated.

To further examine the roles of voltage-gated Ca^{2+} channels, ionomycin (a Ca^{2+} ionophore) was used to facilitate Ca^{2+} entry. However, as the effects of ionomycin are irreversible, a modified protocol was used. Endothelium-denuded mesenteric arteries were depleted of intracellular Ca^{2+} stores as described above, followed by incubation with 1 μM ionomycin for 30 min. Contraction was then induced by readmission of 2.5 mM $CaCl_2$ and a cumulative concentration – response curve to abn-cbd (3 – 30 μM) was obtained. For comparison, the relaxant effect of abn-cbd in vessels stimulated with methoxamine (10 μM) under similar conditions was also determined.

Data and statistical analysis

All relaxation responses are expressed as percentage relaxation of the tone induced by 10 μM methoxamine, 60 mM KCl or 1 μM ionomycin. Values are given as mean $\pm$ s.e.mean and n represents the number of rats. E_{max} represents the maximum effect and pEC_{50} the negative logarithm of the concentration giving 50% of the maximum response; these values were determined directly from individual log concentration – response curves. Unless otherwise stated, statistical analysis was performed by two-way analysis of variance, of the entire concentration – response curves, followed by Bonferroni *post hoc* tests (StatView 4.5 for the Macintosh; Abacus Concepts, Inc., U.S.A.). Student's t-test was also used where appropriate. For contractile responses to $CaCl_2$, data are expressed as percentage of the maximum contraction to $CaCl_2$ in the presence of 10 μM methoxamine or 60 mM KCl. Statistical comparison was performed by Student's paired t-test at individual concentrations. P-values of less than 0.05 were taken as statistically significant.

Drugs

Methoxamine hydrochloride, carbachol, iberiotoxin, charybdotoxin, L-NAME (L-N^G-nitroarginine methyl ester), Rp-cAMPS (Rp-adenosine 3′,5′-cyclic monophosphothioate triethylamine; Sigma, Gillingham, Dorset) and apamin (Calbiochem, Lutterworth, Leicestershire) were dissolved in deionised water. Indomethacin (Sigma) was dissolved in 5% w v^{-1} $NaHCO_3$ solution. Abnormal-cannabidiol (*trans*-4-[3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol; Tocris Cookson, Avonmouth, Bristol) was supplied in 100% ethanol and diluted in distilled water. Capsaicin (Sigma), SR 141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide), SR 144528 (*N*-[(1*S*)-endo-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide; gifts from Sanofi-Synthélabo, Montpellier, France) were dissolved in 100% ethanol. AM 251 (*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide), AM 281 (1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-4-morpholinyl-1*H*-pyrazole-3-carboxamide), AM 630 (6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl) methanone; Tocris Cookson), 18α-glycyrrhetinic acid and ionomycin (Sigma) were dissolved in dimethyl sulphoxide (Sigma). EGTA (ethylene glycol-bis (β-amino ethyl ether) tetraacetic acid; Sigma) was dissolved in Ca^{2+}-free Krebs – Henseleit solution.

Results

Table 1 shows that the increase in tone induced by methoxamine in the test for endothelium at the start of an experiment was not significantly different from the methoxamine contraction obtained in the same vessels after the different treatments and used for the determination of relaxation responses.

Relaxation to abn-cbd in rat small mesenteric artery

Abn-cbd produced concentration-dependent relaxation of endothelium-intact vessels precontracted with methoxamine ($pEC_{50} = 6.2 \pm 0.1$, $E_{max} = 93 \pm 2\%$; $n = 4$; Figure 1). Removal of the endothelium resulted in a six-fold rightward displacement of the concentration – response curve ($pEC_{50} = 5.3 \pm 0.1$,

Table 1 Methoxamine-induced tone in the absence and presence of cannabinoid antagonists, capsaicin, 18α-glycyrrhetinic acid, pertussis toxin, Rp-cAMPS or vehicle

Control	*Tension (1)*	*Treatment*	*Tension (2)*	*n*
With endothelium	14.6±3.4	+SR 141716A (3 μM)	13.9±3.9	4
Without endothelium	14.7±1.9	+SR 141716A (3 μM)	16.1±1.8	4
With endothelium	11.8±1.3	+AM 251 (1 μM)	10.8±1.3	4
With endothelium	12.1±2.0	+AM 281 (1 μM)	12.0±1.9	4
With endothelium	17.4±2.0	+AM 630 (10 μM)	15.7±2.1	4
With endothelium	14.6±3.7	+SR 144528 (1 μM)	11.1±3.9	6
With endothelium	21.7±1.3	+pertussis toxin (400 ng ml^{-1})	19.2±2.0	6
With endothelium	14.7±2.9	+18α-glycyrrhetinic acid (100 μM)	12.7±2.7	5
With endothelium	16.1±2.4	+Rp-cAMPS (100 μM)	17.1±2.7	4
Without endothelium	12.6±3.4	+capsaicin (10 μM)	14.7±3.0	4
With endothelium	17.1±0.4	+dimethyl sulphoxide (0.1% v v^{-1})	16.0±1.7	4
With endothelium	15.7±1.3	+ethanol (0.1% v v^{-1})	15.6±1.2	4

The first value of tension is that obtained in the test for endothelium and the second is that obtained in the same vessels after preconstriction for measurement of relaxation responses. Both responses are to 10 μM methoxamine. All experiments were performed in the presence of 10 μM indomethacin. Data are expressed as mean±s.e. mean. No significant differences from control values were found.

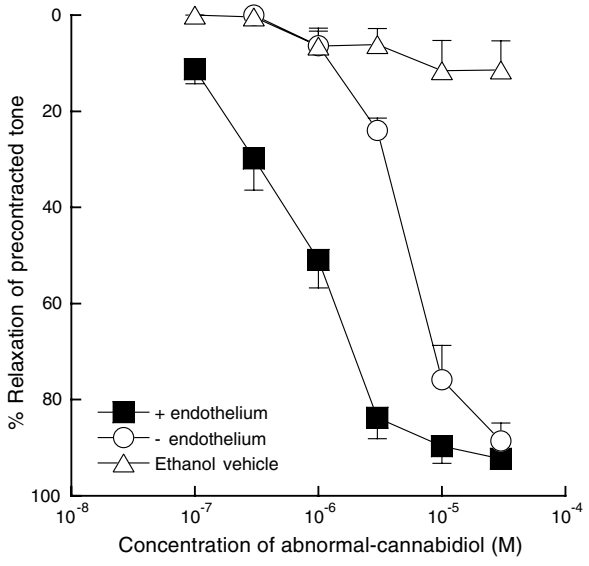

Figure 1 Concentration – response curves for abnormal-cannabidiol (abn-cbd) induced relaxation of methoxamine-induced tone in rat isolated small mesenteric artery in the presence ($n=4$) or absence ($n=4$) of a functional endothelium. Also shown are the effects of the appropriate amounts of vehicle, 0.01 – 0.3% v v^{-1} ethanol ($n=6$). Values are shown as means and vertical lines represent s.e. mean.

$E_{max}=89\pm4\%$; $n=4$; $P<0.01$; Figure 1). Figure 1 also shows that the vehicle (0.01 – 0.3% v v^{-1} ethanol) had little effect on methoxamine-precontracted vessels.

Relaxations to abn-cbd were not significantly different in the presence of 10 μM indomethacin ($pEC_{50}=5.9\pm0.2$, $E_{max}=85\pm4\%$; $n=4$) or in its absence ($pEC_{50}=5.9\pm0.1$, $E_{max}=93\pm1\%$; $n=4$). Capsaicin pretreatment (10 μM), which causes functional desensitisation of the vanilloid receptor system, also had no effect on relaxation induced by abn-cbd in endothelium-denuded vessels (in the presence of capsaicin: $pEC_{50}=5.3\pm0.1$, $E_{max}=82\pm2\%$; $n=4$).

Effects of cannabinoid receptor antagonists on relaxation to abn-cbd

In endothelium-intact vessels, the commonly used CB_1 cannabinoid receptor antagonist SR 141716A (200 nM-3 μM) inhibited relaxation to abn-cbd in a concentration-dependent manner (Table 2; Figure 2a). SR 141716A, at 200 nM, had no significant effect, while, at 1 μM, it shifted the concentration – response curve rightwards approximately eight-fold ($P<0.01$). SR 141716A (3 μM) failed to cause further inhibition of abn-cbd induced relaxation. Figure 2b shows original traces of relaxation to abn-cbd in the absence and presence of SR 141716A (3 μM) in vessels with intact endothelium. In the absence of endothelium, SR 141716A (3 μM) caused small, but significant, inhibition of relaxation to abn-cbd (in the presence of SR 141716A: $pEC_{50}=5.2\pm0.2$, $E_{max}=82\pm5\%$; $n=4$; $P<0.01$; Figure 2c).

In contrast to SR 141716A, another CB_1 receptor antagonist AM 251 (1 μM) only slightly attenuated the relaxation to abn-cbd in endothelium-intact vessels ($P<0.01$; Table 2; Figure 3a). Also, the CB_1 receptor antagonist AM 281 (1 μM) was without any effect (Table 2; Figure 3a). The CB_2 receptor antagonist SR 144528 (1 μM) caused a small rightward displacement of the concentration – response curve for abn-cbd ($P<0.01$; Table 2; Figure 3b), but another CB_2 receptor antagonist AM 630 (10 μM) had no effect on relaxation elicited by abn-cbd (Table 2).

The presence of dimethyl sulphoxide (vehicle for AM 251, AM 281 and AM 630; 0.1% v v^{-1} final bath concentration) or ethanol (vehicle for SR 141716A and SR 144528; 0.1% v v^{-1} final bath concentration) had no significant effects (assessed by analysis of variance) on relaxation to abn-cbd (control: $pEC_{50}=6.2\pm0.1$, $E_{max}=90\pm6\%$; $n=3$; in the presence of dimethyl sulphoxide: $pEC_{50}=6.2\pm0.4$, $E_{max}=95\pm1\%$; $n=4$; in the presence of ethanol: $pEC_{50}=6.2\pm0.1$, $E_{max}=90\pm2\%$; $n=4$).

Effects of abn-cbd on KCl (60 mM)-precontracted vessels

In endothelium-intact vessels precontracted with KCl (60 mM) abn-cbd was about seven-fold less potent as a vasorelaxant

Table 2 Effects of cannabinoid receptor antagonists on relaxation to abn-cbd in endothelium-intact small mesenteric arteries precontracted with methoxamine

Abn-cbd	pEC_{50}	E_{max} (%)	*n*
Control	6.1±0.1	93±2	8
+AM 281 (1 μM)	6.0±0.1	94±1	4
+AM 251 (1 μM)	5.7±0.1	94±2	4*
Control	6.2±0.1	93±2	4
+SR 141716A (200 nM)	6.1±0.1	90±3	4
+SR 141716A (1 μM)	5.3±0.1	82±4	4*
+SR 141716A (3 μM)	5.2±0.2	65±13	4*
Control	6.0±0.1	96±1	6
+SR 144528 (1 μM)	5.9±0.1	84±7	6*
Control	5.9±0.2	89±4	4
+AM 630 (10 μM)	5.7±0.1	91±2	4

All experiments were performed in the presence of 10 μM indomethacin. Data are expressed as mean±s.e.mean. pEC_{50} and E_{max} values were obtained directly from individual log concentration – response curves. *n* represents the number of animals. *Indicates significantly different from control values (two-way analysis of variance; $P<0.01$).

than in methoxamine-precontracted vessels (methoxamine: $pEC_{50}=6.1\pm0.1$, $E_{max}=89\pm2\%$; $n=7$; KCl: $pEC_{50}=5.3\pm0.1$, $E_{max}=83\pm4\%$; $n=7$; $P<0.01$; Figure 4a). In the absence of endothelium, the presence of high extracellular K^+ also attenuated relaxation to abn-cbd, but by less than three-fold (methoxamine: $pEC_{50}=5.4\pm0.1$, $E_{max}=83\pm5\%$; $n=7$; KCl: $pEC_{50}=5.1\pm0.2$, $E_{max}=74\pm8\%$; $n=4$; $P<0.01$; Figure 4a). A trace showing the relaxation induced by abn-cbd in endothelium-intact vessels precontracted with 60 mM KCl is shown in Figure 4b. It is noteworthy that the time course of relaxation is generally slower under KCl-induced tone than that observed in methoxamine-precontracted vessels (cf. Figure 2b).

Effects of L-NAME and K^+ channel blockers on relaxation to abn-cbd

In endothelium-intact vessels, the nitric oxide synthase inhibitor L-NAME (300 μM) alone had no significant effect on relaxation induced by abn-cbd (Table 3; Figure 5a). However, combination of L-NAME (300 μM) with apamin (a blocker of small conductance Ca^{2+}-activated K^+ channels, SK_{Ca}; 50 nM) and charybdotoxin (a blocker of intermediate conductance Ca^{2+}-activated K^+ channels, IK_{Ca}, and large

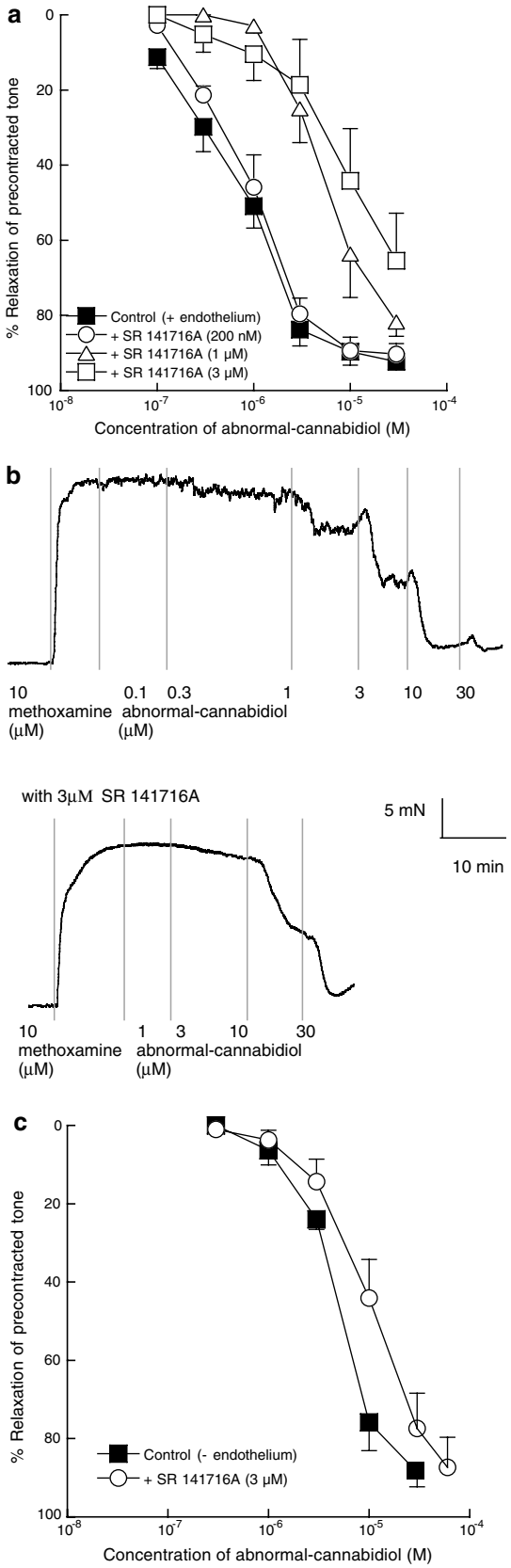

Figure 2 (**a**) Concentration – response curves for abnormal-cannabidiol (abn-cbd) relaxation of methoxamine-induced tone in endothelium-intact rat isolated small mesenteric artery. Relaxation was elicited by abn-cbd alone, and in the presence of 200 nM, 1 μM or 3 μM SR 141716A; $n=4$ for all. Values are shown as means and vertical lines represent s.e. mean. (**b**) Original traces of relaxation to abn-cbd in the absence and presence of 3 μM SR 141716A in separate endothelium-intact vessels are shown. Vertical lines denote addition of drugs at the concentration indicated. (**c**) Concentration – response curves for abn-cbd relaxation of methoxamine-induced tone in endothelium-denuded rat isolated mesenteric artery. Relaxation was elicited by abn-cbd alone, and in the presence of 3 μM SR 141716A $n=4$ for both. Values are shown as means and vertical lines represent s.e.mean.

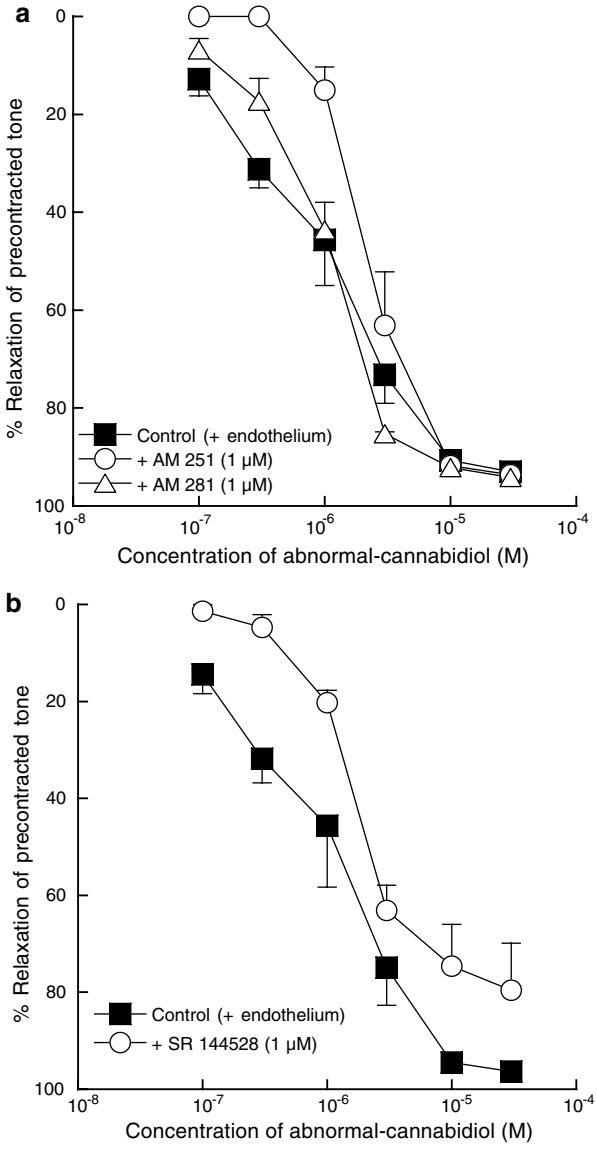

Figure 3 Concentration – response curves for abnormal-cannabidiol (abn-cbd) relaxation of methoxamine-induced tone in endothelium-intact rat isolated small mesenteric artery. (**a**) Relaxation was elicited by abn-cbd alone ($n=8$), and in the presence of 1 μM AM 251 ($n=4$) or 1 μM AM 281 ($n=4$). (**b**) Relaxation was elicited by abn-cbd alone and in the presence of 1 μM SR 144528; $n=6$ for both. Values are shown as means and vertical lines represent s.e.mean.

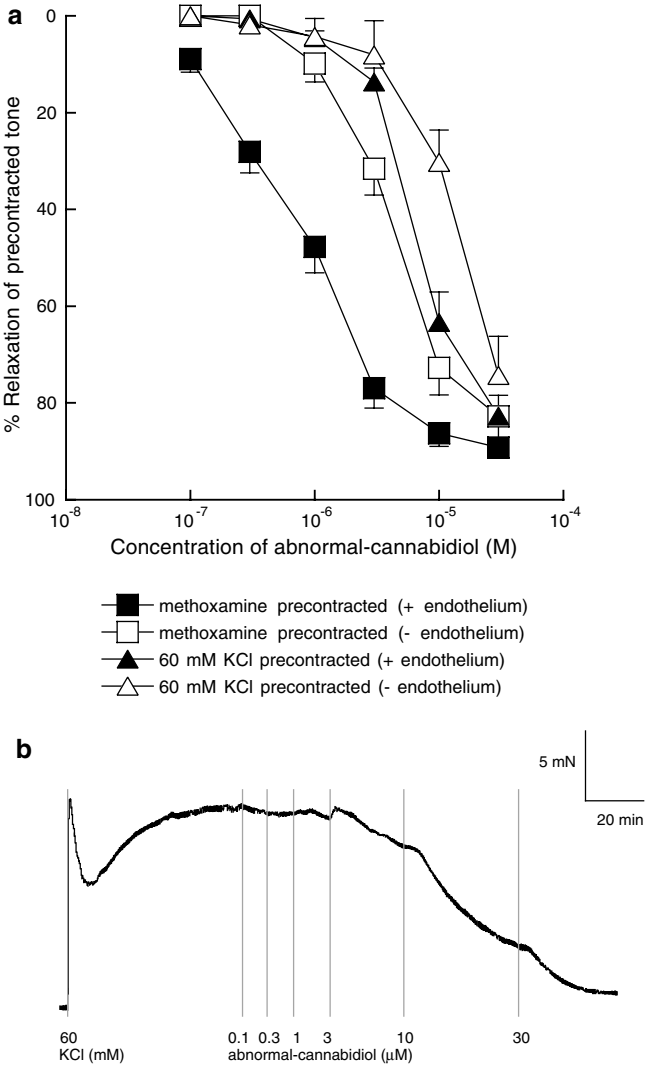

Figure 4 (**a**) Concentration – response curves for abnormal-cannabidiol (abn-cbd) relaxation of either methoxamine- or KCl-induced tone in rat isolated small mesenteric artery. Abn-cbd relaxed methoxamine-precontracted vessels in the presence ($n=7$) or absence ($n=7$) of endothelium. Relaxation to abn-cbd was also observed in 60 mM KCl-precontracted vessels in the presence ($n=7$) or absence ($n=4$) of endothelium. Values are shown as means and vertical lines represent s.e. mean. (**b**) Original recording of the relaxation to abn-cbd in KCl-precontracted, endothelium-intact rat isolated mesenteric artery is shown. Vertical lines denote addition of drugs at the concentration indicated.

conductance Ca^{2+}-activated K^+ channels, BK_{Ca}; 50 nM) shifted the relaxation curve to abn-cbd to the right by approximately four-fold ($P<0.01$; Table 3; Figure 5a). As noted above, the presence of 3 μM SR 141716A inhibited relaxation to abn-cbd, but additional incubation of SR 141716A-treated vessels with L-NAME (300 μM), apamin (50 nM) and charybdotoxin (50 nM) did not cause further inhibition of abn-cbd relaxations (data not shown).

The combination of L-NAME, apamin and iberiotoxin (a blocker of BK_{Ca}; 50 nM) also caused a small rightward shift of abn-cbd relaxation curve (approximately two-fold; $P<0.01$; Figure 5b). Relaxation to abn-cbd was only slightly attenuated by L-NAME plus apamin, most notably at concentrations greater than 1 μM ($P<0.01$; Figure 5b), while combination of L-NAME with either iberiotoxin or charybdotoxin had no significant effects (Table 3).

In endothelium-denuded vessels, the combination of L-NAME, apamin and charybdotoxin had no effect on abn-cbd relaxations (control: $pEC_{50}=5.6\pm0.1$, $E_{max}=75\pm8\%$; $n=3$; in the presence of inhibitors: $pEC_{50}=5.4\pm0.2$, $E_{max}=86\pm7\%$; $n=3$).

Effects of pertussis toxin, a protein kinase A inhibitor and a gap junction inhibitor on relaxation to abn-cbd

All these experiments were performed in endothelium-intact vessels in order to investigate the endothelium-dependent relaxation to abn-cbd. The presence of pertussis toxin

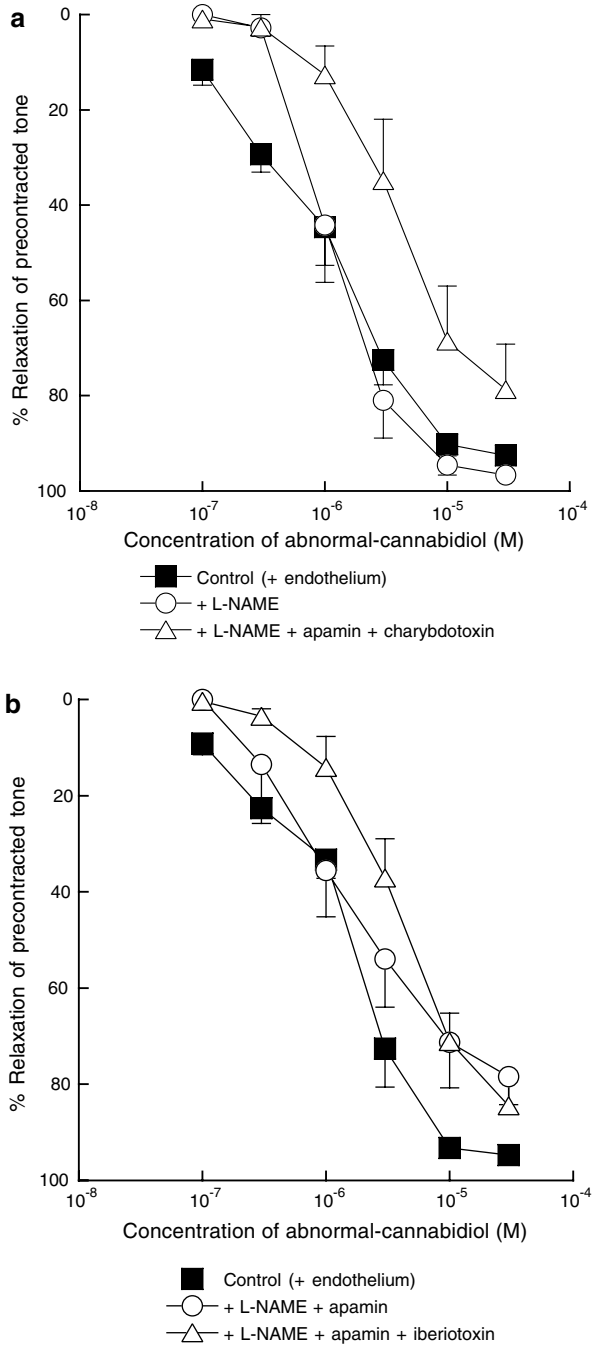

Figure 5 Concentration – response curves for abnormal-cannabidiol (abn-cbd) relaxation of methoxamine-induced tone in endothelium-intact rat isolated mesenteric artery. (**a**) Relaxation was elicited by abn-cbd alone ($n=9$), or in the presence of either 300 μM L-NAME alone ($n=4$) or in the presence of 300 μM L-NAME, 50 nM apamin and 50 nM charybdotoxin ($n=6$). (**b**) Relaxation was elicited by abn-cbd alone ($n=5$), or in the presence of 300 μM L-NAME in combination with 50 nM apamin ($n=4$) or 50 nM apamin and 50 nM iberiotoxin ($n=4$). Values are shown as means and vertical lines represent s.e.mean.

Table 3 Effects of L-NAME and K^+ channel blockers on relaxation to abn-cbd in endothelium-intact small mesenteric arteries precontracted with methoxamine

Abn-cbd	*pEC50*	E_{max} (%)	*n*
Control	6.0±0.1	93±2	9
+L-NAME	5.9±0.1	97±1	4
+L-NAME+apamin+charybdotoxin	5.4±0.1	79±10	6*
Control	5.8±0.1	95±2	5
+L-NAME+apamin	5.9±0.2	78±6	4*
+L-NAME+apamin+iberiotoxin	5.5±0.1	85±6	4*
Control	5.8±0.1	89±2	5
+L-NAME+iberiotoxin	5.8±0.1	88±4	6
+L-NAME+charybdotoxin	5.6±0.1	96±3	4

All experiments were performed in the presence of 10 μM indomethacin. Data are expressed as mean±s.e. mean. pEC_{50} and E_{max} values were obtained directly from individual log concentration – response curves. *n* represents the number of animals. The inhibitors were used at the following concentrations: L-NAME (300 μM), apamin (50 nM), charybdotoxin (50 nM) and iberiotoxin (50 nM). *Indicates significantly different from control values (two-way analysis of variance; $P<0.01$).

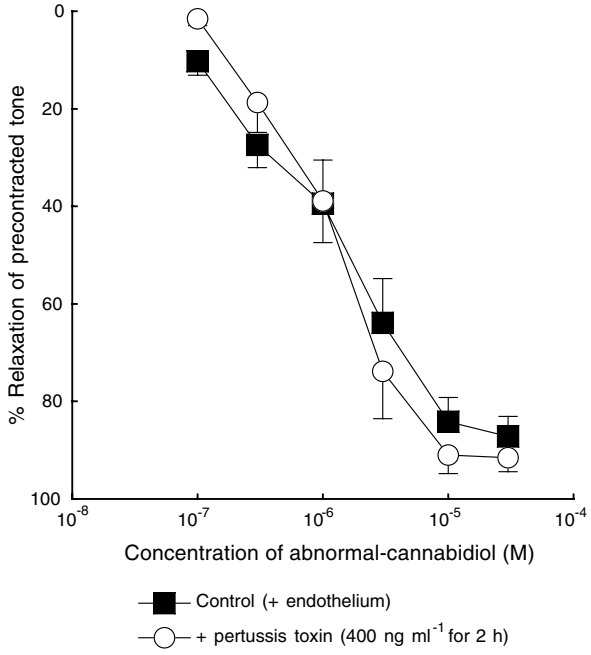

Figure 6 Concentration – response curves for abnormal-cannabidiol (abn-cbd) relaxation of methoxamine-induced tone in endothelium-intact rat isolated small mesenteric artery. Relaxation was elicited by abn-cbd alone or in the presence of 400 ng ml^{-1} pertussis toxin (with 2 h preincubation); $n=6$ for both. Values are shown as means and vertical lines represent s.e.mean.

(400 ng ml^{-1} for 2 h; White & Hiley, 1997), which inhibits $G_{i/o}$ proteins, did not affect relaxation induced by abn-cbd (control: $pEC_{50}=5.9\pm0.2$, $E_{max}=87\pm4\%$; $n=6$; in the presence of pertussis toxin: $pEC_{50}=5.9\pm0.1$, $E_{max}=92\pm3\%$; $n=6$; Figure 6). Similarly, the relaxant effects of abn-cbd were also unaffected by the presence of the protein kinase A inhibitor Rp-cAMPS (100 μM) (control: $pEC_{50}=6.0\pm0.3$, $E_{max}=95\pm2\%$; $n=4$; in the presence of Rp-cAMPS: $pEC_{50}=6.1\pm0.1$, $E_{max}=94\pm1\%$; $n=4$). Inhibition of gap junction function with 100 μM 18α-glycyrrhetinic acid also had

no significant effect on relaxation to abn-cbd (control: $pEC_{50} = 5.7 \pm 0.1$, $E_{max} = 89 \pm 3\%$; $n = 4$; in the presence of 18α-glycyrrhetinic acid: $pEC_{50} = 5.7 \pm 0.2$, $E_{max} = 83 \pm 3\%$; $n = 5$).

Effects of abn-cbd on $CaCl_2$-induced contractions in the absence of extracellular Ca^{2+}

To further examine the role of VGCC in endothelium-independent relaxation induced by abn-cbd, all these experiments were performed in endothelium-denuded vessels which were first depleted of intracellular Ca^{2+} and then stimulated with methoxamine (10 μM) in the absence of extracellular Ca^{2+}. Under these conditions $CaCl_2$ (0.01 – 10 mM) caused concentration-dependent contractions up to 1 – 3 mM and produced relaxation at higher concentrations ($n = 12$). Figure 7a shows that abn-cbd (3 μM) had no significant effect on responses to $CaCl_2$, but increasing its concentration to 10 and 30 μM produced concentration-dependent inhibition of $CaCl_2$-induced contractions ($n = 4$ for all). A trace from a typical experiment is shown in Figure 7b. The presence of 3 μM SR 141716A did not reverse the inhibitory effect of 10 μM abn-cbd (data not shown). In addition, when vessels were stimulated with 60 mM KCl instead of 10 μM methoxamine, abn-cbd (3 and 10 μM) similarly inhibited $CaCl_2$-induced contractions (data not shown).

Figure 8 shows that the relaxant effects of abn-cbd (3 – 30 μM) were greatly reduced when the Ca^{2+} ionophore ionomycin (1 μM), as compared to methoxamine (10 μM), was used to elicit Ca^{2+} entry upon readmission of 2.5 mM $CaCl_2$. All vessels were denuded of endothelium, depleted of intracellular Ca^{2+} stores and initially maintained in the absence of extracellular Ca^{2+} as described in Methods.

Effects of verapamil on methoxamine- and 60 mM KCl-induced tone

The L-type Ca^{2+} channel inhibitor verapamil (1 nM – 10 μM) caused concentration-dependent relaxation of either methoxamine- or 60 mM KCl-precontracted vessels denuded of endothelium, but verapamil was approximately five-fold more potent under KCl-induced tone (methoxamine: $pEC_{50} = 6.4 \pm 0.1$, $E_{max} = 98 \pm 1\%$; $n = 4$; KCl: $pEC_{50} = 7.1 \pm 0.1$, $E_{max} = 97 \pm 3\%$; $n = 4$).

Discussion

This study provides some insights into the means by which abn-cbd causes vasorelaxation in the rat isolated small mesenteric artery. Our results show that endothelium-dependent relaxation to abn-cbd involves activation of K^+ channels via an SR 141716A-sensitive pathway, which is apparently independent of the classical CB_1 and CB_2 cannabinoid receptors. Abn-cbd might also decrease Ca^{2+} entry via voltage-gated Ca^{2+} channels in the vascular smooth muscle by a separate mechanism.

In the rat isolated small mesenteric artery, although the vasorelaxant potency of abn-cbd was reduced by removal of endothelium, the maximum relaxation was unaffected. This is in contrast to the initial report by Járai *et al.* (1999), which showed that denudation greatly inhibited the maximal effect of

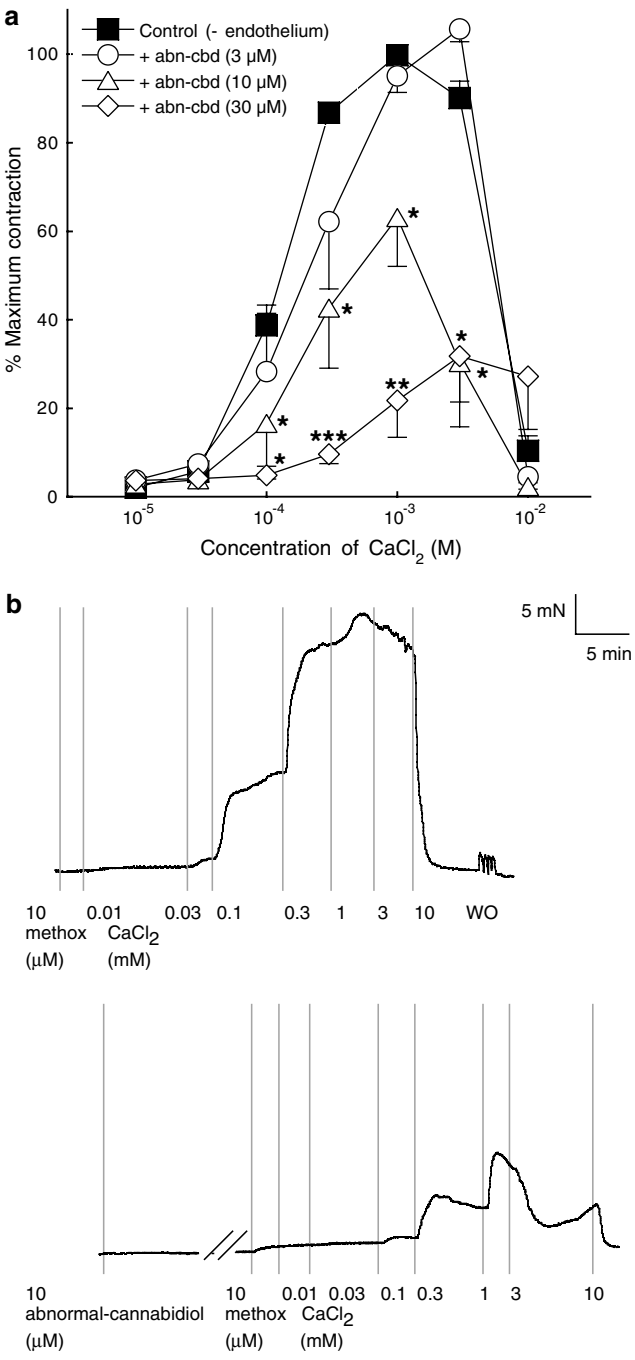

Figure 7 (**a**) Concentration – response curves for $CaCl_2$-induced contractions of methoxamine (10 μM)-stimulated rat isolated small mesenteric arteries depleted of intracellular Ca^{2+} stores with EGTA as described in Methods. All vessels were denuded of endothelium. Contractions to $CaCl_2$ were determined in the absence ($n = 12$) or presence of 3 μM ($n = 4$), 10 μM ($n = 4$) or 30 μM ($n = 4$) abnormal-cannabidiol (abn-cbd) in a given vessel. Control response curves were pooled for clarity. Values are shown as means and vertical lines represent s.e. mean. Statistical comparisons were made by paired *t*-test at individual concentrations. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ indicate significant differences from control values. (**b**) Shows an original recording of $CaCl_2$-induced contractions in the absence and presence of 10 μM abn-cbd. Vertical lines denote addition of drugs at the concentration indicated. WO denotes wash out with Ca^{2+}-free Krebs – Henseleit solution.

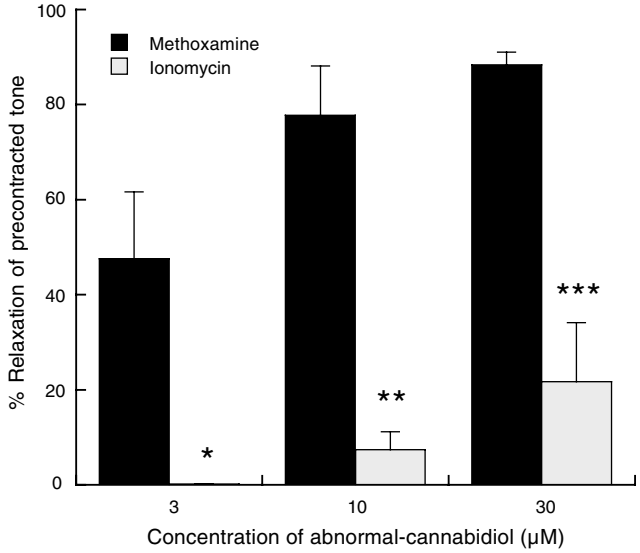

Figure 8 Abnormal-cannabidiol induced relaxation of contractions to 2.5 mM $CaCl_2$ in ionomycin-(1 μM; $n=9$) or methoxamine-(10 μM; $n=4$) stimulated rat isolated small mesenteric artery in the absence of extracellular Ca^{2+}. All vessels were denuded of endothelium and depleted of intracellular Ca^{2+} stores with EGTA as described in Methods. Values are shown as means and vertical lines represent s.e. mean. Statistical comparisons were made by unpaired *t*-test at individual concentrations. $*P<0.05$, $**P<0.01$, $***P<0.001$ indicate significant differences from the corresponding values using methoxamine.

abn-cbd in the rat perfused mesenteric bed preparation. This is likely to be because of a regional effect arising from our use of isolated mesenteric arteries and the relative concentrations of abn-cbd used in the two studies. In fact, we also found that in rat isolated main mesenteric artery, relaxation to abn-cbd was abolished in denuded vessels (Ho & Hiley, unpublished observations). However, it is unlikely that the reduction in potency of abn-cbd is because of damage to the underlying smooth muscle during the rubbing procedure as there was no change in the responsiveness to methoxamine after endothelium denudation. Furthermore, we have previously shown that removal of the endothelium by rubbing with a hair has no effect on the effectiveness of anandamide (White & Hiley, 1997) and, indeed, the potency of the nitrovasodilator s-nitroso-*N*-acetylpenicillamine is enhanced after this procedure (White & Hiley, 1998a).

In endothelium-intact small mesenteric arteries with tone induced by depolarising K^+ solution (60 mM KCl), the vasorelaxant potency of abn-cbd was reduced approximately seven-fold without affecting the maximum relaxation. This indicates that relaxation to abn-cbd is partially mediated by activation of K^+ channels, as high extracellular K^+ solution abolishes the electrochemical gradient for K^+ efflux. Involvement of nitric oxide, which is known to activate K^+ channels, (K_{ATP}, Murphy & Brayden, 1995; BK_{Ca}, Mistry & Garland, 1998) can be excluded, as the nitric oxide synthase inhibitor L-NAME did not significantly affect relaxation to abn-cbd. The combination of L-NAME, apamin (SK_{Ca} inhibitor) and charybdotoxin (blocker of BK_{Ca} and IK_{Ca}) shifted the concentration–response curve of abn-cbd to the right by approximately four-fold, which indicates the involvement of Ca^{2+}-activated K^+ channels. This is consistent with results reported by Járai *et al.* (1999). BK_{Ca} might be involved in mediating the response as charybdotoxin could be substituted, in part, by iberiotoxin (an inhibitor of BK_{Ca}) such that a combination of L-NAME, apamin and iberiotoxin also produced a small, but significant, rightward shift in the abn-cbd concentration–response curve. Although voltage-gated K^+ channels (K_V) are also targets of charybdotoxin, these channels are unlikely to be involved in abn-cbd relaxations as Zygmunt *et al.* (1997) showed that charybdotoxin, up to 300 nM, had no effects on K_V-mediated current. On the other hand, apamin-sensitive K^+ channels might also play a role as L-NAME plus apamin attenuated the maximal relaxant effect of abn-cbd. However, as combined addition of L-NAME and iberiotoxin or charybdotoxin had no significant effects on abn-cbd relaxations, the relative roles of charybdotoxin-, iberiotoxin- and apamin-sensitive K^+ channels remain unclear.

Activation of K^+ channels, nevertheless, is likely to underpin endothelium-dependent relaxation to abn-cbd only because L-NAME, apamin and charybdotoxin failed to inhibit abn-cbd relaxations in endothelium-denuded vessels. This sensitivity of endothelium-dependent relaxation to the combination of apamin and charybdotoxin is a hallmark of agents acting through endothelium-derived hyperpolarizing factors (EDHF; see Busse *et al.*, 2002 for review), suggesting that abn-cbd might act by releasing EDHF. However, the nature of EDHF remains elusive. EDHF might be a diffusible endothelium-derived factor that activates smooth muscle K^+ channels and/or a hyperpolarizing current generated by activation of endothelial K^+ channels, which is transmitted to the smooth muscle via gap junctions. In this study, we showed that the gap junction inhibitor 18α-glycyrrhetinic acid had no significant effect on relaxation induced by abn-cbd despite using a concentration and protocol that has previously been shown to inhibit effects mediated through gap junctions (White & Hiley, 2000). Further investigation is warranted to explore the potential roles of endothelial K^+ channels as well as the involvement of diffusible factors.

Another point to consider is that of production of active metabolites from abn-cbd. Even though *in vivo* metabolism of cannabidiol, the parent compound of abn-cbd, has been studied in detail (Agurell *et al.*, 1986), production of vasoactive metabolites from abn-cbd has not been reported. Nonetheless, in our experiments, relaxation to abn-cbd cannot be attributed to the production of cyclooxygenase products, as abn-cbd relaxation was unaffected by the presence of the cyclooxygenase inhibitor indomethacin.

To examine the role of cannabinoid receptors in relaxation to abn-cbd, the effects of abn-cbd in endothelium-intact vessels were tested in the presence of a range of cannabinoid receptor antagonists. The commonly used cannabinoid antagonist SR 141716A (Felder *et al.*, 1995; Rinaldi-Carmona *et al.*, 1996; Showalter *et al.*, 1996), at 1 μM, caused a rightward displacement (approximately eight-fold) of the concentration–response curve of abn-cbd in endothelium-intact vessels, whereas at 200 nM, it had no significant effect. Increasing the concentration of SR 141716A to 3 μM did not result in further inhibition. In contrast SR 141716A (3 μM) only had a small effect on relaxation induced by abn-cbd in endothelium-denuded vessels. Therefore, SR 141716A has a maximal inhibitory effect on endothelium-dependent relaxation to abn-cbd at concentrations $\leqslant 3\,\mu$M. These concentrations are lower than those at which SR 141716A has been shown to have

overt, noncannabinoid receptor-related effects on endothelium-independent relaxations in rat mesenteric arteries (White & Hiley, 1998c).

It should also be noted that the K_D of SR 141716A, as estimated from the magnitude of shift caused by 1 μM, is about 150 nM which is not consistent with the binding constants at the cloned cannabinoid receptors (CB_1: $K_i = 5.6 - 12.3$ nM, CB_{1A}: $K_i = 43.3$ nM & CB_2: $K_i = 702$ nM; Felder *et al*., 1995; Rinaldi-Carmona *et al*., 1996; Showalter *et al*., 1996). Our observations that other cannabinoid receptor antagonists, used at concentrations more than 100-fold greater than the K_i for their preferred receptors, either had small (AM 251, CB_1 receptor; Lan *et al*., 1999; SR 144528, CB_2 receptor; Rinaldi-Carmona *et al*., 1998; Ross *et al*., 1999) or no effects (AM 281, CB_1 receptor; Gatley *et al*., 1998; AM 630, CB_2 receptor; Ross *et al*., 1999) on abn-cbd relaxations also discounts the involvement of classical cannabinoid receptors. These data are consistent with the results of Járai *et al*. (1999) who found that abn-cbd caused hypotension and relaxation of mesenteric arteries in CB_1 and CB_2 receptor knockout mice and yet these responses were sensitive to SR 141716A. One possible explanation is that abn-cbd activates a SR 141716A-sensitive, but yet-to-be-identified, cannabinoid site in the endothelium of rat mesenteric artery.

Indeed, a putative endothelial abn-cbd receptor, distinct from the classical cannabinoid receptors, has been suggested (Járai *et al*., 1999). This putative site was termed the 'endothelial anandamide receptor' as it was initially proposed to mediate SR 141716A-sensitive mesenteric vasorelaxation to the endocannabinoid anandamide but further studies indicated that it might also recognise abn-cbd (Járai *et al*., 1999; Wagner *et al*., 1999). Results from the present study indicate that this cannabinoid site displays distinct structural requirements as AM 281, AM 251 and SR 144528 are, in fact, structural analogues of the diarylpyrazole SR 141716A (see Howlett *et al*., 2002 for review). Our results, together with the structure – activity relations for vasodilator action of abn-cbd and its analogue O-1602 investigated by Járai *et al*. (1999), provide an initial structural characterisation of the proposed cannabinoid site. As noted above, abn-cbd appears to display regional differences in the contribution of endothelium to its relaxation in rat mesenteric arteries. It is, therefore, interesting to note that while relaxation to anandamide in rat isolated small mesenteric artery is also sensitive to SR 141716A but not to AM 281, it is not affected by removal of the endothelium or the presence of L-NAME, apamin plus charybdotoxin (White & Hiley, 1997; Ho & Hiley, unpublished observations). These results are at odds with studies by Wagner *et al*. (1999) who showed that, in rat perfused mesenteric bed, anandamide produced endothelium-dependent relaxation that was inhibited by SR 141716A. It follows that the endothelial abn-cbd site might play a larger role in anandamide relaxations measured using the perfused bed preparation.

Alternatively, the endothelial abn-cbd site in the rat small mesenteric artery could be distinct from the proposed 'endothelial anandamide receptor', which might be found in other regions of the whole mesenteric bed. In view of the recent proposals that additional cannabinoid receptors distinct from the currently known receptor subtypes might be present in the rat isolated coronary artery (vasorelaxation; White *et al*., 2001; Ford *et al*., 2002) and central nervous system (stimulation of GTPγS binding; Di Marzo *et al*., 2000; Breivogel *et al*., 2001), further investigations are needed to determine if the proposed abn-cbd site is found in other regions of the vasculature.

In addition to its endothelial effects, anandamide can cause endothelium-independent relaxation by stimulating vanilloid VR1 receptors which leads to release of the vasodilator, calcitonin-gene related peptide, from sensory nerves (Zygmunt *et al*., 1999; Ralevic *et al*., 2000; White *et al*., 2001). As the cannabinoid antagonist, SR 141716A can also inhibit VR1 receptor-mediated responses, at least in cultured cells expressing these receptors (De Petrocellis *et al*., 2001), it seems likely that the SR 141716A-sensitive, but endothelium-independent, relaxation to anandamide in the rat small mesenteric artery reflects VR1 receptor-mediated relaxation. It also shows the need for caution when using SR141716A to infer the involvement of cannabinoid receptors in an effect.

Our observation that SR 141716A and a combination of L-NAME, apamin and charybdotoxin had similar inhibitory effects, which are not additive, on abn-cbd relaxation suggests that they share a common pathway. One possibility is that SR 141716A inhibits abn-cbd-induced EDHF production. In fact, SR 141716A (1 μM) has been found to attenuate endothelium-dependent, EDHF-mediated, relaxation to acetylcholine or the Ca^{2+} ionophore A 23187 in mesenteric arteries (rat: White & Hiley, 1997; rabbit: Kagota *et al*., 2001). It is, therefore, possible that SR 141716A inhibits abn-cbd-induced relaxation via receptor-independent mechanisms. Nevertheless, an endothelial site of action is likely as SR 141716A, at a concentration of 1 μM, has no significant effect on macroscopic K^+ current of dissociated smooth muscle cells (Bukoski *et al*., 2002) nor on endothelium-independent relaxation induced by vasodilators such as nitric oxide donors or the BK_{Ca} activator NS 1619 (White & Hiley, 1998c). It is, however, unlikely that SR 141716A inhibits EDHF generation by a nonspecific inhibitory effect on Ca^{2+} handling, as it has no effect on intracellular Ca^{2+} levels of endothelial cells at 1 μM (Mombouli *et al*., 1999). Nevertheless, SR 141716A might exert additional inhibitory actions at higher concentrations. At 10 μM, SR 141716A has been reported to inhibit intercellular communications, possibly by blocking gap junctions (Chaytor *et al*., 1999). This might account for the inhibitory effect of SR 141716A on EDHF-mediated relaxation induced by acetylcholine and A 23187 (White & Hiley, 1997; Kagota *et al*., 2001) but, in the present study, the lack of effect of the gap junction inhibitor 18α-glycyrrhetinic acid on abn-cbd-induced relaxation argues against this possibility. An alternative explanation, as discussed previously, is that SR 141716A antagonises the putative endothelial receptor for abn-cbd and thereby prevents the subsequent activation of apamin- and charybdotoxin-sensitive K^+ channels.

As CB_1 and CB_2 receptors are $G_{i/o}$-protein coupled receptors, the effect of pertussis toxin, which uncouples the interactions of $G_{i/o}$ proteins with their receptors, on relaxation to abn-cbd was tested. Pertussis toxin (400 ng ml^{-1} for 2 h) did not affect the relaxant effects of abn-cbd indicating a limited role of pertussis toxin-sensitive $G_{i/o}$ proteins especially since this lack of effect is unlikely to be due to insufficient treatment as we have found the same protocol is effective at inhibiting acetylcholine-induced relaxation (White & Hiley, 1997). Under certain circumstances cannabinoid receptors might stimulate

G_s proteins in addition to $G_{i/o}$ proteins (Felder *et al*., 1993; Glass & Felder, 1997). However, inhibition of protein kinase A, the main effector of G_s protein activation of adenylyl cyclase, by 100 μM Rp-cAMPS (though we have shown 50 μM Rp-cAMPS to inhibit dibutyryl-cAMP- or forskolin-mediated vasorelaxation, Omar *et al*., 2000) had no effect on abn-cbd relaxations. It therefore seems unlikely that the actions of abn-cbd are mediated through changes in cyclic AMP levels though both cyclic AMP and cyclic GMP inhibit L-type Ca^{2+} currents in vascular smooth muscle cells (Liu *et al*., 1997). Together, our findings suggest that the endothelial component of abn-cbd-induced relaxation involves an SR 141716A-sensitive and Ca^{2+}-activated K^+ channel-dependent pathway, but further studies are required to elucidate the detailed mechanisms involved.

As noted above, in small mesenteric arteries without endothelium, relaxation to abn-cbd was unaffected by L-NAME, apamin and charybdotoxin and was only slightly attenuated by 3 μM SR 141716A. It follows that abn-cbd relaxes endothelium-intact and denuded small mesenteric arteries by distinct mechanisms. In the rat mesenteric artery, activation of VR1 receptors largely explains the endothelium-independent relaxation to anandamide (Zygmunt *et al*., 1999; Ralevic *et al*., 2000; White *et al*., 2001). The parent compound of abn-cbd, cannabidiol, is also an agonist of VR1 receptors (Bisogno *et al*., 2001), but in the present study pretreatment of vessels with capsaicin, which is an agonist of VR1 receptors and causes functional desensitisation of capsaicin-sensitive sensory nerves, had no effect on endothelium-independent relaxation to abn-cbd. Therefore, it is unlikely that an action through VR1 receptors plays a major part in the endothelium-independent vasorelaxation to abn-cbd but direct examination of its effects on VR1 receptors would be needed to show that it cannot activate them. Nevertheless, although abn-cbd and anandamide might activate a common endothelial site, abn-cbd also causes endothelium-independent vasorelaxation by mechanisms distinct from that of anandamide.

Tone induced by depolarising K^+ solution is mediated almost entirely through opening of VGCC (Karaki *et al*., 1997). Therefore, the ability of abn-cbd to retain most of its relaxant effect on denuded vessels precontracted with 60 mM KCl indicates that abn-cbd might in some way inhibit VGCC opening. To provide further evidence for such a mechanism, we examined the effects of abn-cbd on contractions induced by cumulative addition of Ca^{2+} to methoxamine-stimulated vessels, which had been depleted of intracellular Ca^{2+} stores, in the absence of extracellular Ca^{2+}. Under the protocol employed, contractions to Ca^{2+} occur mainly through Ca^{2+} influx following activation of VGCC in the vascular smooth muscle (White & Hiley, 1998c). Abn-cbd (3 – 30 μM) caused potent inhibition of $CaCl_2$-induced contractions, producing up to about 70% reduction in maximal contractions to Ca^{2+}. It was noted that at higher concentrations ($\geqslant$3 mM), $CaCl_2$ produced relaxation that was probably mediated by activation of Ca^{2+} receptors on perivascular sensory nerves (Mupanomunda *et al*., 1998). The presence of abn-cbd had no obvious effects on these $CaCl_2$-induced relaxations. When 60 mM KCl was substituted for 10 μM methoxamine, abn-cbd similarly inhibited $CaCl_2$-induced contractions. The ability of abn-cbd to relax methoxamine- or KCl-precontracted vessels and to inhibit contractions to Ca^{2+} in methoxamine- or KCl-stimulated vessels in the absence of extracellular Ca^{2+} suggests that abn-cbd can inhibit both the initiation and maintenance of contractions and confirms that abn-cbd relaxations do not involve antagonism of α_1-adrenoceptors or IP_3-mediated release of intracellular Ca^{2+} stores.

It is also unlikely that abn-cbd acts by reducing the sensitivity of contractile proteins to Ca^{2+} in vascular smooth muscle cells because relaxation to abn-cbd was greatly inhibited when ionomycin was used to enable Ca^{2+} entry. Ionomycin is a Ca^{2+} ionophore, which causes global elevation of intracellular Ca^{2+} independent of receptor and VGCC activation (Liu & Hermann, 1978). Therefore, we propose that abn-cbd relaxes endothelium-denuded small mesenteric arteries by inhibiting Ca^{2+} influx through VGCC. The L-type Ca^{2+} channel is likely to be the main VGCC involved as the L-type Ca^{2+} channel blocker verapamil inhibits methoxamine- and KCl-induced contractions, as well as $CaCl_2$-induced contraction in methoxamine-stimulated vessels (this study; White & Hiley, 1998c). This mechanism is likely to account for the residual relaxation to abn-cbd in endothelium-intact vessels incubated with the maximally effective concentration of SR 141716A (this study; Járai *et al*., 1999). Interestingly, we also found that in rat isolated main mesenteric arteries precontracted with methoxamine, abn-cbd relaxations were abolished by endothelium removal (Ho & Hiley, unpublished observations). This further argues against an inhibition of Ca^{2+} sensitivity of contractile proteins by abn-cbd. Furthermore, it might also indicate that abn-cbd modulates Ca^{2+} entry through L-type channels by some means that does not involve a direct interaction with the channels since we have evidence that they are also present in the main mesenteric arteries which relax to verapamil under methoxamine-induced tone ($pEC_{50} = 6.0 \pm 0.1$, $E_{max} = 98 \pm 2\%$; $n = 3$) and KCl-induced tone ($pEC_{50} = 6.3 \pm 0.1$, $E_{max} = 98 \pm 1\%$; $n = 4$; Ho & Hiley, unpublished observations). Some potent cannabinoid receptor agonists have been suggested to cause SR 141716A-sensitive inhibition of L-type Ca^{2+} channels (WIN 55, 212-2, Gebremedhin *et al*., 1999; CP 55, 940, White & Hiley, 1998b). However, we found that SR 141716A (3 μM) had very small effects on abn-cbd relaxations and failed to reverse the inhibitory effect of abn-cbd on $CaCl_2$-induced contractions in denuded small mesenteric arteries. Hence, the mechanisms by which abn-cbd modulates L-type Ca^{2+} channels remain to be defined.

In summary, this study has demonstrated that abnormal-cannabidiol (abn-cbd) induces vasorelaxation in rat isolated small mesenteric artery by multiple mechanisms. The endothelium-dependent relaxation involves SR 141716A-sensitive pathways that are distinct from the classical CB_1 and CB_2 cannabinoid receptors and might involve activation of Ca^{2+}-activated K^+ channels. Our results are consistent with the presence of a yet-to-be-identified SR 141716A-sensitive cannabinoid site in the endothelium of rat mesenteric artery. Additionally, we showed that abn-cbd also causes endothelium-independent relaxation by interfering with Ca^{2+} entry via SR 141716A-insensitive pathways.

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References

ADAMS, M.D., EARNHARDT, J.T., MARTIN, B.R., HARRIS, L.S., DEWEY, W.L. & RAZDAN, R.K. (1977). A cannabinoid with cardiovascular activity but no overt behavioural effects. *Experientia,* **33,** 1204 – 1205.

AGURELL, S., HALLDIN, M., LINDGREN, J.E., OHLSSON, A., WIDMAN, M., GILLESPIE, H. & HOLLISTER, L. (1986). Pharmacokinetics and metabolism of delta 1-tetrahydrocannabinol and other cannabinoids with emphasis on man. *Pharmacol. Rev.,* **38,** 21 – 43.

BENOWITZ, N.L. & JONES, R.T. (1974). Cardiovascular effects of prolonged delta-9-tetrahydro-cannabinol ingestion. *Clin. Pharmacol. Ther.,* **18,** 287 – 297.

BISOGNO, T., HANUS, L., DE PETROCELLIS, L., TCHILIBON, S., PONDE, D.E., BRANDI, I., MORIELLO, A.S., DAVIS, J.B., MECHOULAM, R. & DI MARZO, V. (2001). Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br. J. Pharmacol.,* **134,** 845 – 852.

BREIVOGEL, C.S., GRIFFIN, G., DI MARZO, V. & MARTIN, B.R. (2001). Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Mol. Pharmacol*., **60,** 155 – 163.

BUKOSKI, R.D., BÁTKAI, S., JÁRAI, Z., WANG, Y., OFFERTALER, L., JACKSON, W.F. & KUNOS, G. (2002). CB_1 receptor antagonist SR141716A inhibits Ca^{2+}-induced relaxation in CB_1 receptor-deficient mice. *Hypertension,* **39,** 251 – 257.

BUSSE, R., EDWARDS, G., FELETOU, M., FLEMING, I., VANHOUTTE, P.M. & WESTON, A.H. (2002). EDHF: bringing the concepts together. *Trends Pharmacol. Sci.,* **23,** 374 – 380.

CHAYTOR, A.T., MARTIN, P.E., EVANS, W.H., RANDALL, M.D. & GRIFFITH, T.M. (1999). The endothelial component of cannabinoid-induced relaxation in rabbit mesenteric artery depends on gap junctional communication. *J. Physiol.,* **520,** 539 – 550.

DE PETROCELLIS, L., BISOGNO, T., MACCARRONE, M., DAVIS, J.B., FINAZZI-AGRO, A. & DI MARZO, V. (2001). The activity of anandamide at vanilloid VR1 receptors requires facilitated transport across the cell membrane and is limited by intracellular metabolism. *J. Biol. Chem.,* **276,** 12856 – 12863.

DEUTSCH, D.G., GOLIGORSKY, M.S., SCHMID, P.C., KREBSBACH, R.J., SCHMID, H.H.O., DAS, S.K., DEY, S.K., ARREAZA, G., THORUP, C., STEFANO, G. & MOORE, L.C. (1997). Production and physiological actions of anandamide in the vasculature of the rat kidney. *J. Clin. Invest.,* **100,** 1538 – 1546.

DI MARZO, V., BREIVOGEL, C.S., TAO, Q., BRIDGEN, D.T., RAZDAN, R.K., ZIMMER, A.M. & MARTIN, B.R. (2000). Levels, metabolism and pharmacological activity of anandamide in CB_1 cannabinoid receptor knockout mice: evidence for non-CB_1, non-CB_2 receptor-mediated actions of anandamide in mouse brain. *J. Neurochem.,* **75,** 2434 – 2444.

ELLIS, E.F., MOORE, S.F. & WILLOUGHBY, K.A. (1995). Anandamide and delta 9-THC dilation of cerebral arterioles is blocked by indomethacin. *Am. J. Physiol.,* **269,** H1859 – H1864.

FACCI, R., DAL TOSO, R., ROMANELLO, S., BURUANI, A., SKAPER, S.D. & LEON, A. (1995). Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. *Proc. Natl. Acad. Sci. U.S.A.,* **92,** 3376 – 3380.

FELDER, C.C., BRILEY, E.M., AXELROD, J., SIMPSON, J.T., MACKIE, K. & DEVANE, W.A. (1993). Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction. *Proc. Natl. Acad. Sci. U.S.A.,* **90,** 7656 – 7660.

FELDER, C.C. & GLASS, M. (1998). Cannabinoid receptors and their endogenous agonists. *Annu. Rev. Pharmacol. Toxicol.,* **38,** 179 – 200.

FELDER, C.C., JOYCE, K.E., BRILEY, E.M., MANSOURI, J., MACKIE, K., BLOND, O., LAI, Y., MA, A.L. & MITCHELL, R.L. (1995). Comparison of the pharmacology and signal transduction of the human cannabinoid CB_1 and CB_2 receptors. *Mol. Pharmacol.,* **48,** 443 – 450.

FORD, W.R., HONAN, S.A., WHITE, R. & HILEY, C.R. (2002). Evidence of a novel site mediating anandamide-induced negative inotropic and coronary vasodilatator responses in rat isolated hearts. *Br. J. Pharmacol.,* **135,** 1191 – 1198.

GATLEY, S.J., LAN, R., VOLKOW, N.D., PAPPAS, N., KING, P., WONG, C.T., GIFFORD, A.N., PYATT, B., DEWEY, S.L. & MAKRIYANNIS, A. (1998). Imaging the brain marijuana receptor: development of a radioligand that binds to cannabinoid CB_1 receptors *in vivo*. *J. Neurochem.,* **70,** 417 – 423.

GEBREMEDHIN, D., LANGE, A.R., CAMPBELL, W.B., HILLARD, C.J. & HARDER, D.R. (1999). Cannabinoid CB_1 receptor of rat cerebral arterial muscle functions to inhibit L-type Ca^{2+} channel current. *Am. J. Physiol.,* **276,** H2085 – H2093.

GLASS, M. & FELDER, C.C. (1997). Concurrent stimulation of cannabinoid CB_1 and dopamine D_2 receptors augments cAMP accumulation in striatal neurons: evidence for a G_s linkage to the CB_1 receptor. *J. Neurosci.,* **17,** 5327 – 5333.

HANUS, L., BREUER, A., TCHILIBON, S., SHILOAH, S., GOLDENBERG, D., HOROWITZ, M., PERTWEE, R.G., ROSS, R.A., MECHOULAM, R. & FRIED, E. (1999). HU308: a specific agonist for CB_2, a peripheral cannabinoid receptor. *Proc. Natl. Acad. Sci. U.S.A.,* **25,** 14228 – 14233.

HILLARD C.J. (2000). Endocannabinoids and vascular function. *J. Pharmacol. Exp. Ther.,* **294,** 27 – 32.

HOWLETT, A.C., BARTH, F., BONNER, T.I., CABRAL, G., CASELLAS, P., DEVANE, W.A., FELDER, C.C., HERKENHAM, M., MACKIE, K., MARTIN, B.R., MECHOULAM, R. & PERTWEE, R.G. (2002). International Union of Pharmacology. XXVII. Classification of Cannabinoid Receptors. *Pharmacol. Rev.,* **54,** 161 – 202.

JÁRAI, Z., WAGNER, J.A., VARGA, K., LAKE, K.D., COMPTON, D.R., MARTIN, B.R., ZIMMER, A.M., BONNER, T.I., BUCKLEY, N.E., MEZEY, E., RAZDAN, R.K., ZIMMER, A. & KUNOS, G. (1999). Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB_1 or CB_2 receptors. *Proc. Natl. Acad. Sci. U.S.A.,* **96,** 14136 – 14141.

KAGOTA, S., YAMAGUCHI, Y., MAKAMURA, K., SUGUIRA, T., WAKU, K. & KUNITOMO, M. (2001). 2-Arachidonylglycerol, a candidate of endothelium-derived hyperpolarizing factor. *Eur. J. Pharmacol.,* **415,** 233 – 238.

KARAKI, H., OZAKI, H., HORI, M., MITSUSAITO, M., AMINO, K.I., HARADA, K.I., MIYAMOTO, S., NAKAZAWA, H., WON, K.J. & SATO, K. (1997). Calcium movements, distribution, and functions in smooth muscle. *Pharmacol. Rev.,* **49,** 157 – 230.

LAKE, K.D., COMPTON, D.R., VARGA, K., MARTIN, B.R. & KUNOS, G. (1997). Cannabinoid-induced hypotension and bradycardia in rats is mediated by CB_1-like cannabinoid receptors. *J. Pharmacol. Exp. Ther.,* **281,** 1030 – 1037.

LAN, R., LIU, Q., FAN, P., LIN, S., FERNANDO, S.R., MCCALLION, D., PERTWEE, R. & MAKRIYANNIS, A. (1999). Structure – activity relationships of pyrazole derivatives as cannabinoid receptor antagonists. *J. Med. Chem.,* **42,** 769 – 776.

LIU, C. & HERMANN, T.E. (1978). Characterization of ionomycin as a calcium ionophore. *J. Biol. Chem.,* **253,** 5892 – 5894.

LIU, H., XIONG, Z. & SPERELAKIS, N. (1997). Cyclic nucleotides regulate the activity of L-type calcium channels in smooth muscle cells from rat portal vein. *J. Mol. Cell. Cardiol*., **29,** 1411 – 1421.

MANSBACH, R.S., ROVETTI, C.C., WINSTON, E.N. & LOWE, J.A. (1996). Effects of the cannabinoid CB_1 receptor antagonist SR141716A on the behaviour of pigeons and rats. *Psychopharmacology,* **124,** 315 – 332.

MATSUDA, L.A., LOLAIT, S.J., BROWNSTEIN, M.J., YOUNG, A.C. & BONNER, T.I. (1990). Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature,* **346,** 561 – 564.

MISTRY, D.K. & GARLAND, C.J. (1998). Nitric oxide (NO)-induced activation of large-conductance Ca^{2+}-dependent K^+ channels (BK_{Ca}) in smooth muscle cells isolated from the rat mesenteric artery. *Br. J. Pharmacol.,* **124,** 1131 – 1140.

MOMBOULI, J.V., SCHAEFFER, G., HOLZMANN, S., KOSTNER, G.M. & GRAIER, W.F. (1999). Anandamide-induced mobilization of cytosolic Ca^{2+} in endothelial cells. *Br. J. Pharmacol.,* **126,** 1593 – 1600.

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

MUPANOMUNDA, M.M., WANG, Y. & BUKOSKI, R.D. (1998). Effect of chronic sensory denervation on Ca^{2+}-induced relaxation of

isolated mesenteric resistance arteries. *Am. J. Physiol.,* **274,** H1655 – H1661.

MURPHY, M.E. & BRAYDEN, J.E. (1995). Nitric oxide hyperpolarizes rabbit mesenteric arteries via ATP-sensitive potassium channels. *J. Physiol.*, **486,** 47 – 58.

OMAR, R., BOTTRILL, F.E., HILEY, C.R. & WHITE, R. (2000). Interaction of cyclic cAMP modulating agents with levcromakalim in the relaxation of rat isolated mesenteric artery. *Eur. J. Pharmacol.*, **401,** 85 – 96.

PRATT, P.F., HILLARD, C.J., EDGEMOND, W.S. & CAMPBELL, W.B. (1998). *N*-arachidonylethanolamide relaxation of bovine coronary artery is not mediated by CB_1 cannabinoid receptor. *Am. J. Physiol.,* **274,** H375 – H381.

RALEVIC, V., KENDALL, D.A., RANDALL, M.D., ZYGMUNT, P.M., MOHAVED, P. & HÖGESTÄTT, E.D. (2000). Vanilloid receptors on capsaicin-sensory nerves mediated relaxation to methanandamide in the rat isolated mesenteric arterial bed and small mesenteric arteries. *Br. J. Pharmacol.,* **130,** 1483 – 1488.

RINALDI-CARMONA, M., BARTH, F., MILLAN, J., DEROCQ, J.M., CASELLAS, P., CONGY, C., OUSTRIC, D., SARRAN, M., BOUABOULA, M., CALANDRA, B., PORTIER, M., SHIRE, D., BRELIERE, J.C. & LE FUR, G. (1998). SR 144528, the first potent and selective antagonist of the CB_2 cannabinoid receptor. *J. Pharmacol. Exp. Ther.,* **284,** 644 – 650.

RINALDI-CARMONA, M., CALANDRA, B., SHIRE, D., BOUABOULA, M., OUSRTIC, D., BARTH, F., CASELLAS, P., FERRARA, P. & LE FUR, G. (1996). Characterisation of two cloned human CB_1 cannabinoid receptor isoforms. *J. Pharmacol. Exp. Ther.,* **278,** 871 – 878.

ROSS, R.A., BROCKIE, H.C., STEVENSON, L.A., MURPHY, V.L., TEMPLETON, F., MAKRIYANNIS, A. & PERTWEE, R.G. (1999). Agonist-inverse agonist characterization at CB_1 and CB_2 cannabinoid receptors of L759633, L759656 and AM630. *Br. J. Pharmacol.,* **126,** 665 – 672.

SHIRE, D., CARILLOM, C., KAGHAD, M., CALANDRA, B., RINALDI-CARMONA, M., LE FUR, G., CAPUT, D. & FERRARA, P. (1995). An amino-terminal variant of the central cannabinoid receptor resulting from alternative splicing. *J. Biol. Chem.,* **270,** 3276 – 3731.

SHOWALTER, V.M., COMPTON, D.R., MARTIN, B.R. & ABOOD, M.E. (1996). Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB_2): identification of cannabinoid receptor subtype selective ligands. *J. Pharmacol. Exp. Ther.,* **278,** 989 – 999.

VARGA, K., LAKE, K., MARTIN, B.R. & KUNOS, G. (1995). Novel antagonist implicates the CB_1 cannabinoid receptor in the hypotensive action of anandamide. *Eur. J. Pharmacol.,* **278,** 279 – 283.

WAGNER, J.A., VARGA, K., JÁRAI, Z. & KUNOS, G. (1999). Mesenteric vasodilation mediated by endothelial anandamide receptors. *Hypertension,* **33,** 429 – 434.

WHITE, R. & HILEY, C.R. (1997). A comparison of EDHF-mediated and anandamide-induced relaxations in the rat isolated mesenteric artery. *Br. J. Pharmacol.,* **122,** 1573 – 1584.

WHITE, R. & HILEY, C.R. (1998a). Modulation of relaxation to levcromakalim by *S*-nitroso-*N*-acetylpenicallamine (SNAP) and 8-bromo cyclic GMP in the rat isolated mesenteric artery. *Br. J. Pharmacol.,* **124,** 1219 – 1226.

WHITE, R. & HILEY, C.R. (1998b). The actions of some cannabinoid receptor ligands in rat isolated mesenteric artery. *Br. J. Pharmacol.,* **125,** 533 – 541.

WHITE, R. & HILEY, C.R. (1998c). The actions of the cannabinoid receptor antagonist, SR 141716A, in the rat isolated mesenteric artery. *Br. J. Pharmacol.,* **125,** 689 – 696.

WHITE, R. & HILEY, C.R. (2000). Hyperpolarisation of rat mesenteric endothelial cells by ATP-sensitive K^+ channel openers. *Eur. J. Pharmacol.,* **397,** 279 – 290.

WHITE, R., HO, W.-S.V., BOTTRILL, F.E., FORD, W.R. & HILEY, C.R. (2001). Mechanisms of anandamide-induced vasorelaxation in rat isolated coronary arteries. *Br. J. Pharmacol.,* **134,** 921 – 929.

ZYGMUNT, P.M., EDWARDS, G., WESTON, A.H., LARSSON, B., HÖGESTÄTT, E.D. (1997). Involvement of voltage-dependent potassium channels in the EDHF-mediated relaxation of rat hepatic artery. *Br. J. Pharmacol.,* **121,** 141 – 149.

ZYGMUNT, P.M., PETERSSON, J., ANDERSSON, D.A., CHUANG, H., SØRGÅRD, M., DI MARZO, V., JULIUS, D. & HÖGESTÄTT, E.D. (1999). Vanilloid receptors on sensory nerves mediate vasodilator action of anandamide. *Nature,* **400,** 452 – 457.

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