ELSEVIER

European Journal of Pharmacology 418 (2001) 117–125

ejp

www.elsevier.nl/locate/ejphar

Cannabinoid inhibition of capsaicin-sensitive sensory neurotransmission in the rat mesenteric arterial bed

Vera Ralevic *, David A. Kendall

School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, UK

Received 27 November 2000; received in revised form 15 March 2001; accepted 23 March 2001

Abstract

The present study investigated whether cannabinoids can modulate neurotransmission mediated by capsaicin-sensitive sensory nerves in the rat isolated mesenteric arterial bed. Sensory neurogenic vasorelaxation mediated by electrical field stimulation was concentration-dependently attenuated by HU210 (0.1–3 μM), a cannabinoid receptor agonist (from 62 ± 8.3% to 6 ± 2.1% at 3 μM HU210). HU210 had no effect on relaxation to exogenous calcitonin gene-related peptide, indicating a prejunctional action. The action of HU210 (1 μM) was not affected by LY320135 (1 μM) or SR144528 (1 μM), cannabinoid CB_1 and CB_2 receptor antagonists, respectively. SR141716A (0.01–1 μM), a cannabinoid CB_1 receptor antagonist, concentration-dependently augmented vasorelaxation to electrical field stimulation, but had no effect on responses to calcitonin gene-related peptide and capsaicin, indicating a possible role of endogenous cannabinoids in sensory neurotransmission in rat mesenteric arteries. These data show that the cannabinoid receptor agonist HU210 inhibits prejunctionally sensory neurotransmission in rat mesenteric arteries and that this action is independent of cannabinoid CB_1- or CB_2-like receptors.

Keywords: Sensory neurotransmission; Cannabinoid; Cardiovascular system; Mesenteric artery

1. Introduction

Capsaicin-sensitive sensory nerves are widely distributed in the cardiovascular system (Maggi and Meli, 1988). They contain a number of different coexisting neurotransmitters, principal among which are calcitonin gene-related peptide and substance P (Maggi and Meli, 1988; Kawasaki et al., 1988; Fujimori et al., 1989). Sensory nerves are activated by a variety of mechanical and chemical stimuli that present potential challenges to homeostasis. Physiological responses to the actions of released sensory neurotransmitter include vasodilatation and bradycardia. Fine-tuning of perivascular sensory neurotransmission can be achieved by prejunctional modulation of neurotransmitter release, and this is effected by a variety of agents including adenosine (Rubino et al., 1993), opioid peptides (Ralevic et al., 1994), neuropeptide Y (Kawasaki et al., 1991; Li and Duckles, 1991), noradrenaline (Kawasaki et al., 1990) and calcitonin gene-related peptide (Nuki et al., 1994).

Cannabinoids are a novel class of vasoactive compound that can have pronounced effects on the cardiovascular system, including bradycardia, hypotension (Varga et al., 1995; Lake et al., 1997), and relaxation of a variety of blood vessels (Randall et al., 1996; White and Hiley, 1998a,b; Jarai et al., 1999; Zygmunt et al., 1999). Two main types of cannabinoid receptors, both G protein-coupled, have been cloned and characterized: cannabinoid CB_1 receptors are found mainly in the central and peripheral nervous systems, and cannabinoid CB_2 receptors are found mainly in the periphery in association with immune cells (Randall and Kendall, 1998; Pertwee, 1999). There is, however, functional evidence for the expression of both cannabinoid CB_1 and CB_2 receptors on the peripheral terminals of autonomic and myenteric neurones, activation of which mediates inhibition of neurotransmitter release (Ishac et al., 1996; Malinowska et al., 1997; Pertwee, 1999). In addition, cannabinoid CB_1 receptors have been shown to mediate inhibition of primary afferent neurotransmission in the spinal cord (Walker et al., 1999), cannabinoid CB_1 receptor messenger RNA has been lo-

* Corresponding author. Tel.: +44-115-970-9480; fax: +44-115-970-9259.

E-mail address: vera.ralevic@nottingham.ac.uk (V. Ralevic).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)00940-2

calised in dorsal root ganglion cells (Hohmann and Herkenham, 1999a), and there is evidence of trafficking of cannabinoid CB_1 receptors from the dorsal root ganglion to peripheral sensory nerves (Hohmann and Herkenham, 1999b). There is also recent evidence for the presence of both cannabinoid CB_1 and CB_2 receptors on dorsal root ganglion neurones in culture (Ross et al., 2001).

The aim of the present study was to investigate whether cannabinoids can modulate sensory neurotransmission in blood vessels. The role of cannabinoids with respect to perivascular sensory neurotransmission may, however, be complex, as the cannabinoids anandamide and methanandamide can act at vanilloid VR1 receptors on sensory nerves to cause release of calcitonin gene-related peptide and vasorelaxation (Zygmunt et al., 1999; Ralevic et al., 2000). Moreover, endogenous cannabinoid has been detected in dorsal root ganglion cells and peripheral nerves (Huang et al., 1999), and there is pharmacological evidence that anandamide may be released by Ca^{2+} from perivascular sensory nerves (Ishioka and Bukoski, 1999). The rat isolated mesenteric arterial bed was used; in this vascular preparation, activation of sensory nerves by electrical field stimulation causes a release of calcitonin gene-related peptide and vasorelaxation (Kawasaki et al., 1988; Fujimori et al., 1989). We tested the effects of (11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol) HU210, a cannabinoid receptor agonist, and different cannabinoid receptor antagonists, on sensory neurogenic vasorelaxation evoked by electrical field stimulation. HU210 was chosen for study as it is inactive at the vanilloid VR1 receptor (Zygmunt et al., 1999). The effects of HU210 on vasorelaxant responses to exogenous calcitonin gene-related peptide and the sensory neurotoxin capsaicin were additionally investigated. A preliminary account of some of these findings has been reported previously (Ralevic and Kendall, 2000).

2. Materials and methods

2.1. Mesenteric arterial bed preparation

Male Wistar rats (250–300 g) were killed by decapitation after exposure to CO_2. Mesenteric beds were isolated and perfused via the superior mesenteric artery as described previously (Ralevic et al., 1994). Preparations were mounted on a stainless steel grid (7×5 cm) in a humid chamber and perfused at a constant flow rate of 5 ml min^{-1} (pump model 7554-30, Cole-Parmer Instrument, Chicago, IL, USA) with Krebs' solution (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, $CaCl_2$ 2.52 and glucose 7.8, gassed with 95% O_2–5% CO_2 and maintained at 37°C. Guanethidine (5 μM) was added to block sympathetic neurotransmission and, after 30 min, methoxamine (3–40 μM) was added to preconstrict the preparations (by 40–80 mm Hg above baseline). Electrical field stimulation (2–12 Hz, 0.1 ms, 60 V, 30 s) was applied with a Grass S9D stimulator (Grass Instrument, Quincy, MA, USA). Responses were measured as changes in perfusion pressure (mm Hg) (pressure transducer: P23XL, Viggo-Spectramed, Oxnard, CA, USA), and recorded on a polygraph (model 7D, Grass Instrument).

2.2. Experimental protocol

Three consecutive relaxant response curves to electrical field stimulation at 2–12 Hz, "EFS control", "EFS I" and "EFS II", were generated in the preconstricted mesenteric arterial beds. The first response curve acted as a control. HU210 (0.1–3 μM) was then added to the perfusate and, after 15 min, response curves EFS I and EFS II were generated. Only a single concentration of HU210 was tested per preparation. The effects on sensory neurotransmission of cannabinoid receptor antagonists alone, or on inhibition by HU210 of responses to EFS I and EFS II, were investigated by adding these to the perfusate before generating EFS control. In separate experiments, the effect of HU210 (1 μM, 30-min equilibration) on responses to calcitonin gene-related peptide (0.5–50 pmol) and capsaicin (0.0005–50 pmol) was investigated. The effect of SR141716A (1 μM) on relaxations to bolus doses (50 μl injection proximal to the preparation) of calcitonin gene-related peptide, capsaicin, ADP and sodium nitroprusside was additionally investigated. Dimethyl sulphoxide at 0.03% did not affect responses to electrical field stimulation.

2.3. Drugs

SR141716A (*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide) was from RBI (NIMH synthesis programme No. 1MH 30003). LY320135 [6-methoxy-2-(4-methoxyphenyl)benzo[*b*]-thien-3-yl][4-cyanophenyl]methanone) was from Pfizer. HU210 (11-hydroxy-dimethylheptyl-Δ^8-tetrahydrocannabinol) and methanandamide were from Tocris Cookson (Bristol, UK). SR144528 (*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) was from Sanofi (Montpellier, France). ADP, capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), calcitonin gene-related peptide, methoxamine (hydrochloride), and sodium nitroprusside were from Sigma (Poole, Dorset, UK). Guanethidine (Ismelin) was from Alliance Pharmaceuticals (Chippenham, Wiltshire, UK). SR141716A and SR144528 were made up as 10 mM stock solutions in ethanol. LY320135, capsaicin and HU210 were dissolved as stock solutions of 10 mM in dimethyl sulphoxide. All other drugs were dissolved in distilled water.

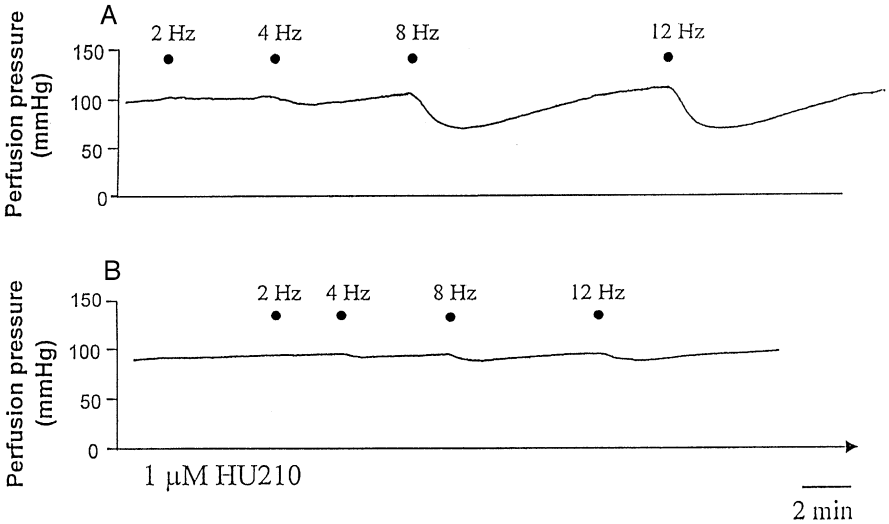

Fig. 1. Representative trace showing the effect of HU210 on frequency-dependent sensory neurogenic vasorelaxation of the rat isolated mesenteric arterial bed. Electrical field stimulation (2–12 Hz, 0.1 ms, 60 V, 30 s) evoked frequency-dependent vasorelaxation and is shown in: (A) control conditions, (B) the presence of HU210 (1 μM). The perfusate contained guanethidine (5 μM) to block sympathetic neurotransmission and methoxamine (5 μM) to preconstrict the preparation. Relaxation to electrical field stimulation was attenuated by HU210.

2.4. Data analysis

Vasorelaxant responses (mm Hg) of the mesenteric arterial beds were expressed as percentage relaxation of the methoxamine-induced increase in tone above baseline. Data were compared by Student's *t* test and analysis of variance (ANOVA). A value of $P < 0.05$ was taken to indicate a statistically significant difference. R_{max} = maximal relaxation. F_{50} is the stimulation frequency (Hz) required to elicit a response that is half of the maximal relaxation. pD_2 is the dose required to elicit a half maximal relaxation.

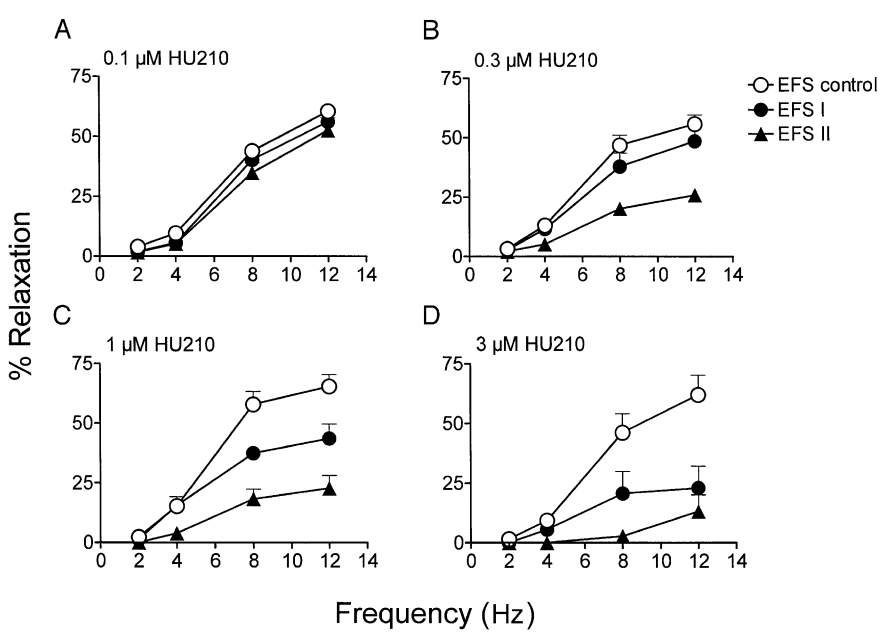

Fig. 2. Inhibition by HU210 of sensory neurotransmission is time and concentration-dependent. Three consecutive frequency-dependent relaxation–response curves to electrical field stimulation (EFS, 2–12 Hz, 0.1 ms, 60 V, 30 s) (EFS control, EFS I and EFS II) were generated in each rat isolated mesenteric arterial bed preparation. EFS control is the response curve in the absence of agents, and EFS I and EFS II were generated in the presence of HU210 (0.1–3 μM). (A) 0.1 μM HU210 ($n = 6$), (B) 0.3 μM HU210 ($n = 4$–9), (C) 1 μM HU210 ($n = 6$–7), (D) 3 μM HU210 ($n = 4$). Data are presented as means and bars indicate S.E.M.

3. Results

3.1. Effect of HU210 on vasorelaxant responses to electrical field stimulation

Electrical field stimulation (2–12 Hz, 0.1 ms, 60 V, 30 s) evoked frequency-dependent relaxations of the mesenteric arterial beds (Figs. 1 and 2), and these responses were reproducible when generated on three consecutive occasions (EFS control, EFS I and EFS II) under control conditions. The vasorelaxations are known to be due principally to the motor actions of calcitonin gene-related peptide released from sensory nerves. Each frequency–response curve was generated in approximately 30 min. The first frequency response curve acted as control. The cannabinoid receptor agonist HU210 (0.1–3 μM) attenuated relaxations to the subsequent second and third frequency response curves (EFS I and EFS II) in a time- and concentration-dependent manner. There was a greater effect of HU210 on responses to EFS II than to EFS I (Figs. 1 and 2). Maximal relaxation (R_{max}) of EFS I was unaffected by 0.1 μM HU210 and 0.3 μM HU210, but was reduced by 1 μM HU210 (from $65 \pm 5.1\%$ EFS control to $44 \pm 6\%$, $P < 0.05$) and 3 μM HU210 (from $62 \pm 8.3\%$ to $23 \pm 9.2\%$, $P < 0.01$). R_{max} of EFS II was unaffected by 0.1 μM HU210 ($53 \pm 5.1\%$) but was attenuated by 0.3 μM HU210 (to $26 \pm 2.2\%$, $P < 0.001$), 1 μM HU210 (to $23 \pm 5.3\%$, $P < 0.001$) and 3 μM HU210 (to $6 \pm 2.1\%$, $P < 0.001$). HU210 did not affect the sensitivity of relaxations to electrical field stimulation. HU210 (0.1–1 μM) had no significant effect on the tone of the preparations, although at 3 μM HU210 there appeared to be a very slow decrease in tone over the course of the experiment.

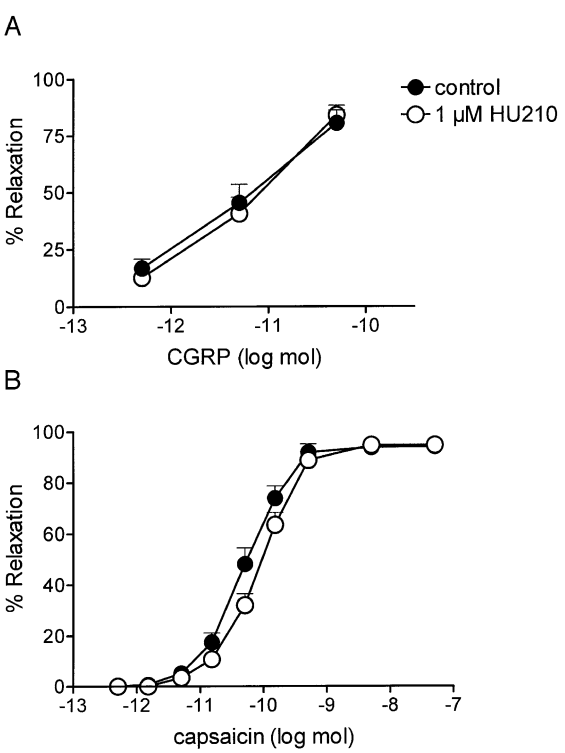

Fig. 3. Effect of HU210 on vasorelaxant responses to calcitonin gene-related peptide and capsaicin of the rat isolated mesenteric arterial bed. Relaxant responses of rat isolated perfused mesenteric arterial beds to (A) calcitonin gene-related peptide (CGRP); (B) capsaicin, in the absence ($n = 8$) and presence of 1 μM HU210 ($n = 6$). Data are presented as means and bars indicate S.E.M.

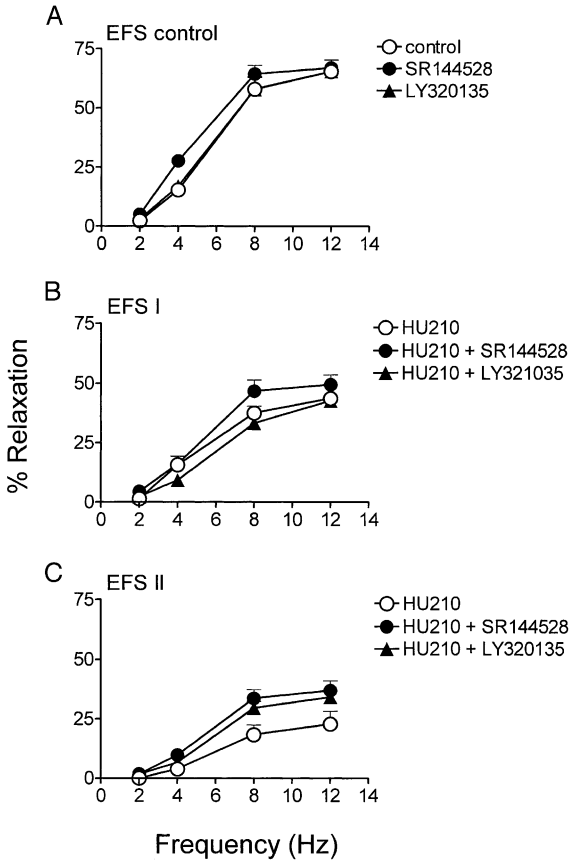

Fig. 4. Effects of the cannabinoid receptor antagonists SR144528 and LY320135 on inhibition by HU210 of sensory neurotransmission in the rat isolated mesenteric arterial bed. The effects of SR 144528 (1 μM, $n = 6$) or LY 320135 (1 μM, $n = 6$) on inhibition by HU210 (1 μM) of relaxant responses to electrical field stimulation (2–12 Hz) of the mesenteric arterial bed were investigated. Three consecutive frequency response curves (EFS control, EFS I and EFS II) were constructed in each preparation over 30 min each. (A) EFS control: control responses generated in the absence of drugs, in the presence of SR144528 alone, and in the presence of LY320135 alone. (B) EFS I: responses generated in the presence of HU210 alone, in the presence of HU210 and SR144528, and in the presence of HU210 and LY320135. (C) EFS II: responses generated in the presence of HU210 alone, in the presence of HU210 and SR144528, and in the presence of HU210 and LY320135. Data are presented as means and bars indicate S.E.M.

3.2. Effect of HU210 on vasorelaxant responses to exogenous calcitonin gene-related peptide and capsaicin

In order to determine whether HU210 was acting pre- or postjunctionally, its effects on vasorelaxant responses to exogenous calcitonin gene-related peptide, the principal

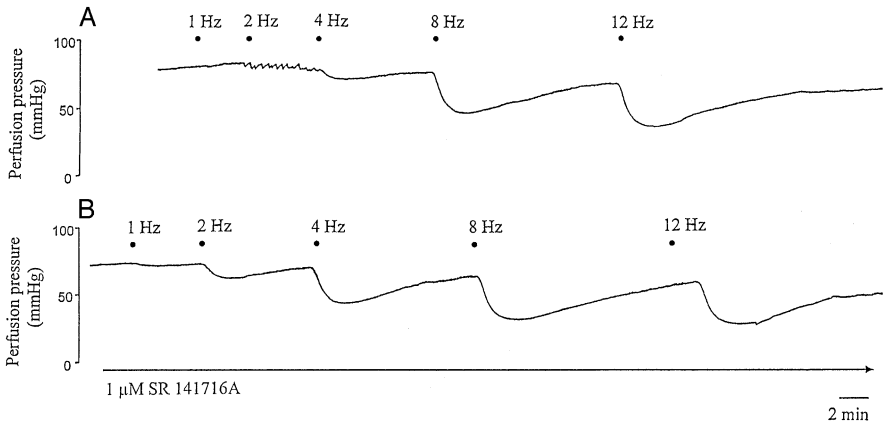

Fig. 5. Representative trace showing augmentation by SR141716A of sensory neurotransmission in the rat isolated mesenteric arterial bed. Representative trace showing frequency-dependent vasorelaxation of a rat mesenteric arterial bed to electrical field stimulation (2–12 Hz) is shown in the absence (A) and presence (B) of SR141716A (1 μM). The perfusate contained guanethidine (5 μM) to block sympathetic neurotransmission and methoxamine (5 μM) to preconstrict the preparation. Relaxation was augmented in the presence of SR 141716A.

motor neurotransmitter of sensory nerves was investigated. HU210 (1 μM) had no significant effect on dose-dependent relaxations to calcitonin gene-related peptide (0.5–50 pmol), indicating that it was acting prejunctionally (Fig. 3). Vasorelaxation response curves to capsaicin (0.5 pmol–50 nmol) were different in the absence and presence of HU210 (ANOVA; $P < 0.05$), although there was no significant difference in the R_{max} or pD_2 values (Fig. 3).

3.3. Effect of LY320135, SR144528 and SR141716A on inhibition by HU210 of vasorelaxant responses to electrical field stimulation

We investigated whether the action of HU210 was mediated by a cannabinoid CB_1 or CB_2 receptor using selective cannabinoid receptor antagonists. Neither LY320135 (1 μM), a selective cannabinoid CB_1 receptor antagonist, nor SR144528 (1 μM), a selective cannabinoid CB_2 receptor antagonist, affected inhibition by HU210 (1 μM) of responses to electrical field stimulation (Fig. 4). R_{max} values for EFS control were $65 \pm 5.1\%$ in the absence of drugs; $67 \pm 2.8\%$ in the presence of SR144528 alone; $65 \pm 4.8\%$ in the presence of LY320135 alone. R_{max} values for EFS I were: $44 \pm 6.0\%$, HU210 alone; $50 \pm 4.1\%$, HU210 + SR144528; $43 \pm 2.4\%$, HU210 + LY320135. R_{max} values for EFS II were: $23 \pm 5.3\%$, HU210 alone; $38 \pm 3.7\%$, HU210 + SR144528; $35 \pm 2.0\%$, HU210 + LY320135. LY320135 (1 μM; $n = 6$) and SR144528 (1 μM; $n = 6$) alone had no significant effects on the tone of the preparations, although LY320135 at 10 μM caused a decrease in tone ($n = 4$).

SR141716A, a selective cannabinoid CB_1 receptor antagonist, at a concentration (0.03 μM) which had no effect on sensory neurotransmission had no effect on inhibition by HU210 (0.3 μM) of relaxations to electrical field stimulation ($n = 6$).

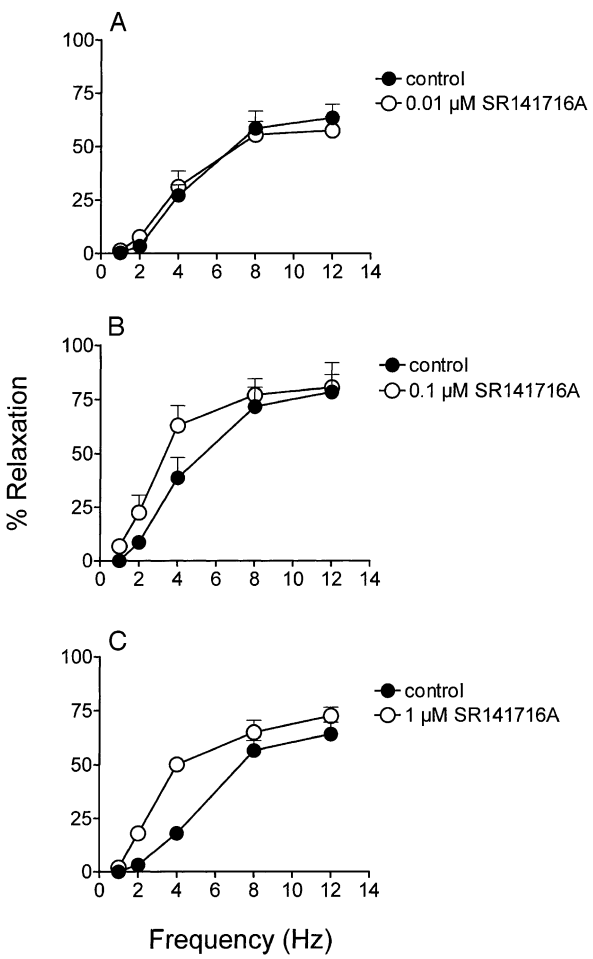

Fig. 6. Effect of SR141716A (0.01–1 μM) on frequency-dependent sensory neurogenic relaxation to electrical field stimulation (2–12 Hz, 0.1 ms, 60 V, 30 s) of the rat isolated mesenteric arterial bed. Symbols show responses in the absence (●) and presence (○) of SR141716A ($n = 4$–8). Data are presented as means and bars indicate S.E.M. SR141716A augmented neurogenic vasorelaxations at 0.1 μM ($P < 0.05$) and 1 μM ($P < 0.01$) causing a leftward shift in the response curves.

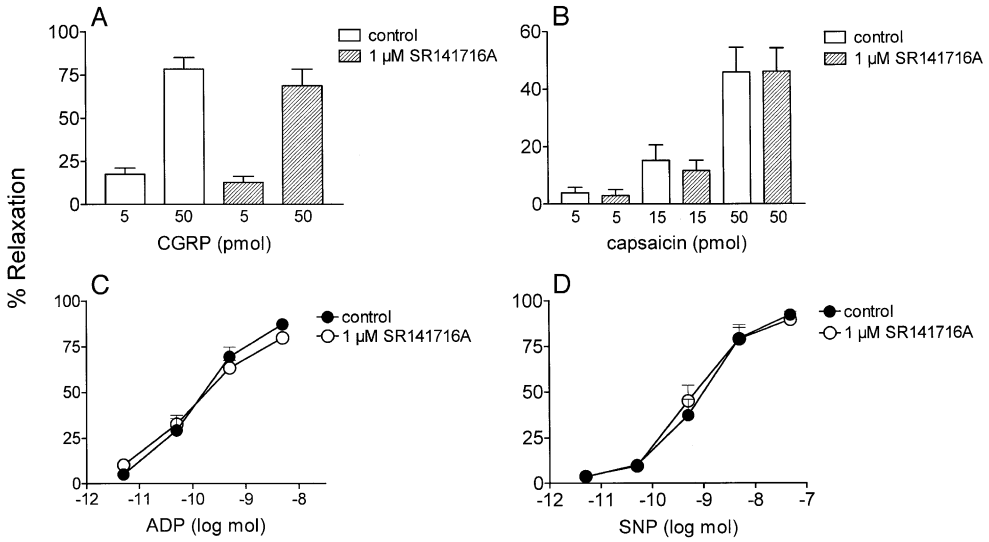

Fig. 7. Effect of SR141716A on vasorelaxant responses to capsaicin and sodium nitroprusside in the rat isolated mesenteric arterial bed. Vasorelaxant responses of rat isolated mesenteric arterial beds to (A) calcitonin gene-related peptide (CGRP); (B) capsaicin (5–50 pmol); (C) ADP (0.005–5 nmol); (D) sodium nitroprusside (SNP; 0.005–50 nmol), in the absence and presence of SR141716A (1 μM; $n = 6$). Data are presented as means and bars indicate S.E.M. There was no difference in relaxations in the absence and presence of SR141716A.

3.4. Effect of SR141716A, LY320135 and SR144528 on vasorelaxant responses to electrical field stimulation

SR141716A (0.01–1 μM) concentration-dependently augmented frequency-dependent vasorelaxation to electrical field stimulation, resulting in a leftward shift in the relaxation–response curves (Figs. 5 and 6). The F_{50} values were 4.4 ± 0.59 Hz in the absence, and 2.8 ± 0.28 Hz in the presence, of 0.1 μM SR141716A ($P < 0.05$), and 5.4 ± 0.2 Hz without, and 3.0 ± 0.1 Hz with, 1 μM SR141716A ($P < 0.01$) (Fig. 6). The lowest concentration of SR141716A tested (0.01 μM) had no significant effect on sensory neurotransmission. At none of the concentrations tested did SR141716A significantly affect the maximal relaxation to electrical field stimulation. SR141716A (0.01–1 μM) had no effect on the tone of the preparations.

Neither LY320135 (1 μM; $n = 6$) nor SR144528 (1 μM; $n = 4$) alone had an effect on neurogenic relaxation to electrical field stimulation. LY320135 at 10 μM, however, caused a fall in tone of the preparations and virtually abolished sensory neurogenic relaxations ($n = 4$), an effect that was not observed with the same concentration of vehicle.

3.5. Effect of SR1417161A on vasorelaxant responses to calcitonin gene-related peptide, capsaicin, ADP and sodium nitroprusside

In order to determine whether the effect of SR141716A was mediated pre- or postjunctionally, we investigated its effects on a range of vasorelaxant agents. SR141716A (1 μM) had no significant effect on dose-dependent vasorelaxations to calcitonin gene-related peptide (5 and 50 pmol), capsaicin (5–50 pmol), ADP (0.005–5 nmol) and sodium nitroprusside (0.005–50 nmol) (Fig. 7).

4. Discussion

The present study has demonstrated clearly for the first time cannabinoid inhibition of sensory neurotransmission in blood vessels. The cannabinoid receptor agonist HU210 inhibited, prejunctionally, vasorelaxant responses mediated by electrical field stimulation of sensory nerves in the rat isolated mesenteric arterial bed. Moreover, augmentation of relaxations to electrical field stimulation by SR141716A, a selective cannabinoid CB_1 receptor antagonist, indicates a possible role of endogenous cannabinoids in the local modulation of blood flow in the mesentery.

HU210 was clearly a potent inhibitor of sensory neurogenic relaxation to electrical field stimulation, but had no effect on relaxation to exogenous calcitonin gene-related peptide, indicating that its action is prejunctional. There is evidence that cannabinoid CB_1 receptors are coupled to the activation of inwardly rectifying K^+ channels and negatively to N-type and P/Q-type Ca^{2+} channels through $G_{i/o}$ proteins (Pertwee, 1999; Sullivan, 1999), and HU210 inhibition of sensory neurotransmission may operate through a similar mechanism. There was a small but significant inhibition of responses to capsaicin which is likely to be prejunctional given the lack of effect of HU210 on responses to calcitonin gene-related peptide. The action of HU210 was observed to be greater with time, which may be due to slow diffusion of this highly lipophilic compound from the lumen to perivascular sen-

sory nerves in the adventitia. Inhibition of sensory neurotransmission is not unique to HU210 as its effects are mimicked by WIN55,212 and CP55,940, two structurally dissimilar cannabinoid receptor agonists (unpublished observations). None of these cannabinoids are agonists at the vanilloid VR1 receptor (Zygmunt et al., 1999), indicating that their actions are mediated independently of VR1. Interestingly, anandamide and methanandamide are agonists at vanilloid VR1 receptors, causing release of sensory neurotransmitter and vasorelaxation (Ralevic et al., 2000; Zygmunt et al., 1999), which indicates that different cannabinoids can have quite different effects on sensory neurotransmission. The physiological significance of this remains to be determined.

Micromolar concentrations of HU210 were required to produce an effect, which does not correspond to its known affinity (nM) for cannabinoid receptors in a range of biochemical assays (see Pertwee, 1993), at endogenous cannabinoid CB_1 receptors on neurones in the mouse vas deferens and guinea-pig small intestine (Pertwee et al., 1992), and at cloned human cannabinoid CB_1 and CB_2 receptors (Felder et al., 1995). Hence, the actions of HU210 in the mesenteric arterial bed may be independent of CB_1 and CB_2 receptors, although the tendency of HU210 to accumulate in lipids could lead to an underestimation of potency in the mesenteric arterial bed. Indeed, the concentration of HU210 used is in the same range as that used by others to describe postjunctional cannabinoid receptors in isolated blood vessels (White and Hiley, 1998a). The inhibitory effect of HU210 was not blocked by the antagonists LY3201345, SR144528 and SR141716A, which further suggests an action independent of cannabinoid CB_1 and CB_2 receptors. This was unexpected given that trafficking of cannabinoid CB_1 receptors from the dorsal root ganglion to peripheral nerves has been shown (Hohmann and Herkenham, 1999b). It is possible that a novel cannabinoid CB_1-like receptor, sensitive to HU210, is expressed on capsaicin-sensitive sensory nerves in rat mesenteric arteries. Indeed, a non-CB_1/CB_2 vascular receptor has been reported to mediate cannabinoid-induced mesenteric vasodilatation (Jarai et al., 1999), indicating that current information about the number and subtypes of cannabinoid receptors may be incomplete. Nonetheless, this study indicates that cannabinoids can act as inhibitory modulators of sensory neurotransmission in mesenteric blood vessels.

SR141716A, a potent and selective cannabinoid CB_1 receptor antagonist with nanomolar affinity for the cannabinoid CB_1 receptor and micromolar affinity for the cannabinoid CB_2 receptor (Rinaldi-Carmona et al., 1994; Pertwee, 2000), augmented sensory neurogenic vasorelaxation to electrical field stimulation. SR141716A has similarly been shown to facilitate C-fibre nociceptive responses of dorsal neurones in rat (Chapman, 1999). In the mesenteric arterial bed, this action was shown to be prejunctional, as relaxations to sodium nitroprusside and calcitonin gene-related peptide were not affected by SR141716A, indicating a possible role of endogenous cannabinoids in modulation of perivascular sensory neurotransmission in rat mesenteric arteries. The most likely source of the endogenous cannabinoids is the sensory nerves themselves. Like classical neurotransmitters, cannabinoids are released from neurones following membrane depolarization and Ca^{2+} influx into the cell, but they are thought to be synthesized on demand, from the phospholipase D-catalysed hydrolysis of a phospholipid precursor, *N*-arachidonyl-phosphatidylethanolamine (Di Marzo et al., 1994, 1998). The newly synthesised cannabinoid is then released from the neurone by passive (and possibly also facilitated) diffusion. Thus, augmentation of sensory neurotransmission by SR141716A in the rat mesenteric arterial bed may be due to block of cannabinoid receptors, and antagonism of the inhibitory action of endogenous cannabinoids released during electrical field stimulation.

LY320135, a selective cannabinoid CB_1 receptor antagonist, and SR144528, a selective cannabinoid CB_2 receptor antagonist, however, did not mimic the effect of SR141716A. LY320135 and SR144528 have nanomolar affinity for cannabinoid CB_1 and CB_2 receptors so it is unlikely that an insufficiently high concentration (1 μM) was used to block cannabinoid actions in the present study. Thus, receptors other than cannabinoid CB_1 and CB_2 receptors may mediate augmentation of sensory neurotransmission by SR141716A. This is in line with our finding that the effect of HU210 on sensory neurotransmission is not blocked by SR141716A and the other cannabinoid CB_1 and CB_2 receptor antagonists. There is also a body of evidence that SR141716A is a potent inverse agonist at constitutively active cannabinoid CB_1 receptors (Bouaboula et al., 1997; MacLennan et al., 1998; Pan et al., 1998; Coutts et al., 2000). It is noteworthy that SR141716A was effective in the present study at concentrations well below those at which non-specific actions have been reported (10 μM and greater) (Chataigneau et al., 1988; White and Hiley, 1998a,b).

In a recent study carried out in rat isolated mesenteric branch arteries, Ishioka and Bukoski (1999) showed that the cannabinoid receptor antagonist SR141716A attenuated vasorelaxant responses mediated by anandamide and by Ca^{2+} acting on sensory nerves. They proposed that anandamide is a hyperpolarising vasodilator compound that is released by sensory nerves in response to Ca^{2+} receptor activation. Neurogenic vasorelaxation mediated by electrical stimulation of the mesenteric arterial bed is virtually abolished by the calcitonin gene-related peptide receptor antagonist calcitonin gene-related peptide (8–37) (Han et al., 1990). Hence, it is possible that the different stimuli used in the two studies evoke a release of different proportions of classical neurotransmitter together with anandamide from sensory nerves. Our studies are, however, in agreement that anandamide could be released from sensory nerves, although we suggest that under the conditions of

the present study it may act as a neuromodulator rather than contributing to the postjunctional response. Ishioka and Bukoski suggested that inhibition by SR141716A of Ca^{2+} responses was due to inhibition of the postjunctional actions of anandamide, released from sensory nerves. The estimated IC_{50} value of 500 nM for SR141716A-mediated inhibition of Ca^{2+}-induced sensory neurogenic relaxation led these investigators to suggest that a CB_2-like receptor is involved in anandamide- and Ca^{2+}-mediated vasorelaxation (Ishioka and Bukoski, 1999). Our conclusion that SR141716A appeared to be acting prejunctionally is drawn from the fact that it had no effect on responses to exogenous calcitonin gene-related peptide.

There is considerable recent interest in the possible clinical use of cannabinoid compounds (Baker et al., 2000; Galve-Roperh et al., 2000), and some success has been found with the use of cannabinoids to control spasticity and tremor in an animal model of multiple sclerosis (Baker et al., 2000). It seems logical that if they are to be used in man, we need to know more about the possible actions of cannabinoids on the cardiovascular system. In this respect, the present study provides novel data showing a role of cannabinoids as inhibitory modulators of perivascular sensory neurotransmission.

In conclusion, this study shows clearly that sensory neurotransmission in blood vessels can be potently modulated by cannabinoids as the selective cannabinoid receptor agonist HU210 inhibited, prejunctionally, capsaicin-sensitive sensory neurotransmission in the rat mesenteric arterial bed. This effect appears to be mediated independently of cannabinoid CB_1- or CB_2-like receptors. Moreover, augmentation of sensory neurogenic vasorelaxation by the cannabinoid receptor antagonist SR141716A indicates a possible role of endogenous cannabinoids in modulation of sensory neurotransmission in rat mesenteric arteries. These data indicate a novel role for cannabinoids as inhibitory modulators of sensory neurotransmission in the mesentery.

Acknowledgements

We are grateful to the Royal Society for financial support.

References

Baker, D., Pryce, G., Croxford, J., Brown, P., Pertwee, R.G., Huffman, J.W., Layward, L., 2000. Cannabinoids control spasticity and tremor in a multiple sclerosis model. Nature 404, 84–87.

Bouaboula, M., Perrachon, S., Canant, X., Rinaldi-Carmona, M., Portier, M., Barth, F., Calandra, B., Pecceu, F., Lupker, J., Maffrand, J.-P., Le Fur, G., Casellas, P., 1997. A selective inverse agonist for central cannabinoid receptors inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1: evidence for a new model of receptor/ligand interactions. J. Biol. Chem. 272, 22330–22339.

Chapman, V., 1999. The cannabinoid CB_1 receptor antagonist, SR141716A, selectively facilitates nociceptive responses of dorsal neurones in the rat. Br. J. Pharmacol. 127, 1765–1767.

Chataigneau, T., Feletou, M., Thollon, C., Villeneuve, N., Vilaine, J.-P., Duhault, J., Vanhoutte, P.M., 1988. Cannabinoid CB_1 receptor and endothelium-dependent hyperpolarization in guinea-pig carotid, rat mesenteric and porcine coronary arteries. Br. J. Pharmacol. 123, 968–974.

Coutts, A.A., Brewster, N., Ingram, T., Razdan, R.K., Pertwee, R.G., 2000. Comparison of novel cannabinoid partial agonists and SR141716A in the guinea-pig small intestine. Br. J. Pharmacol. 129, 645–652.

Di Marzo, V., Fontana, A., Cadas, H., Scinelli, S., Cimino, G., Schwartz, J.-C., Piomelli, D., 1994. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature 372, 686–691.

Di Marzo, V., Melck, D., Bisogno, T., De Petrocellis, L., 1998. Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. Trends Neurosci. 21, 521–528.

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.

Fujimori, A., Saito, A., Kimura, S., Watanabe, T., Uchiyama, Y., Kawaski, H., Goto, K., 1989. Neurogenic vasodilation and release of calcitonin gene-related peptide (CGRP) from perivascular nerves in the rat mesenteric artery. Biochem. Biophys. Res. Commun. 165, 1391–1398.

Galve-Roperh, I., Sánchez, C., Cortés, M.L., Gómez Del Pulgar, T., Izquierdo, M., Guzmán, M., 2000. Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. Nat. Med. 6, 313–319.

Han, S.-P., Naes, L., Westfall, T.C., 1990. Inhibition of periarterial nerve stimulation-induced vasodilation of the mesenteric arterial bed by CGRP (8–37) and CGRP receptor desensitization. Biochem. Biophys. Res. Commun. 168, 786–791.

Hohmann, A.G., Herkenham, M., 1999a. Localization of central cannabinoid CB_1 receptor messenger RNA in neuronal subpopulations of rat dorsal root ganglia: a double-label in situ hybridization study. Neuroscience 90, 923–931.

Hohmann, A.G., Herkenham, M., 1999b. Cannabinoid receptors undergo axonal flow in sensory nerves. Neuroscience 92, 1171–1175.

Huang, S.M., Strangman, N.M., Walker, J.M., 1999. Liquid chromatographic-mass spectrometric measurement of the endogenous cannabinoid 2-arachidonylglycerol in the spinal cord and peripheral nervous system. Acta Pharmacol. Sinica 20, 1098–1102.

Ishac, E.J.N., Jiang, L., Lake, K.D., Varga, K., Abood, M.E., Kunos, G., 1996. Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB_1 receptors on peripheral sympathetic nerves. Br. J. Pharmacol. 118, 2023–2028.

Ishioka, N., Bukoski, R.D., 1999. A role for *N*-arachidonylethanolamine (Anandamide) as the mediator of sensory nerve-dependent Ca^{2+}-induced relaxation. J. Pharmacol. Exp. Ther. 289, 245–250.

Jarai, 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.

Kawasaki, H., Takasaki, K., Saito, A., Goto, K., 1988. Calcitonin gene-related peptide acts as a novel vasodilator neurotransmitter in mesenteric resistance vessels of the rat. Nature 335, 165–167.

Kawasaki, H., Nuki, C., Saito, A., Takasaki, K., 1990. Adrenergic modulation of calcitonin gene-related peptide (CGRP)-containing nerve-mediated vasodilation in the rat mesenteric resistance vessel. Brain Res. 506, 287–290.

Kawasaki, H., Nuki, C., Saito, A., Takasaki, K., 1991. NPY modulates neurotransmission of CGRP-containing vasodilator nerves in rat mesenteric arteries. Am. J. Physiol. 261, H683–H690.

Lake, K.D., Martin, B.R., Kunos, G., Varga, K., 1997. Cardiovascular

effects of anandamide in anaesthetized and conscious normotensive and hypertensive rats. Hypertension 29, 1204–1210.

Li, Y., Duckles, S.P., 1991. Differential effects of neuropeptide Y and opioids on neurogenic responses of the perfused rat mesentery. Eur. J. Pharmacol. 195, 365–372.

Maclennan, S.J., Reynen, P.H., Kwan, J., Bonhaus, D.W., 1998. Evidence for inverse agonism of SR141716A at human recombinant cannabinoid CB_1 and CB_2 receptors. Br. J. Pharmacol. 124, 619–622.

Maggi, C.A., Meli, A., 1988. The sensory-efferent function of capsaicin-sensitive nerves. Gen. Pharmacol. 19, 1–43.

Malinowska, B., Godlewski, G., Bucher, B., Schliker, E., 1997. Cannabinoid CB_1 receptor-mediated inhibition of the neurogenic vasopressor response in the pithed rat. Naunyn-Schmiedeberg's Arch. Pharmacol. 356, 197–202.

Nuki, C., Kawasaki, H., Takasaki, K., Wada, A., 1994. Pharmacological characterization of presynaptic calcitonin gene-related peptide (CGRP) receptors on CGRP-containing vasodilator nerves in rat mesenteric resistance arteries. J. Pharmacol. Exp. Ther. 268, 59–64.

Pan, X., Ikeda, S.R., Lewis, D.L., 1998. SR141716A acts as an inverse agonist to increase neuronal voltage-dependent Ca^{2+} currents by reversal of tonic CB_1 cannabinoid receptor activity. Mol. Pharmacol. 54, 1064–1072.

Pertwee, R.G., 1993. The evidence for the existence of cannabinoid receptors. Gen. Pharmacol. 24, 811–824.

Pertwee, R.G., 1999. Evidence for the presence of CB_1 cannabinoid receptors on peripheral neurones and for the existence of neuronal non-CB_1 cannabinoid receptors. Life Sci. 65, 597–605.

Pertwee, R.G., 2000. Cannabinoid receptor ligands: clinical and neuropharmacological considerations, relevant to future drug discovery and development. Exp. Opin. Invest. Drugs 9, 1–19.

Pertwee, R.G., Stevenson, L.A., Elrick, D.B., Mechoulam, R., Corbett, A.D., 1992. Inhibitory effects of certain enantiomeric cannabinoids in the mouse vas deferens and the myenteric plexus preparation of guinea-pig small intestine. Br. J. Pharmacol. 105, 980–984.

Ralevic, V., Kendall, D.A., 2000. Cannabinoid modulation of capsaicin-sensitive primary afferent neurotransmission in the rat isolated mesenteric arterial bed. J. Auton. Nerv. Syst. 82, 49.

Ralevic, V., Rubino, A., Burnstock, G., 1994. Prejunctional modulation of sensory-motor nerve mediated vasodilatation of the rat mesenteric arterial bed by opioid peptides. J. Pharmacol. Exp. Ther. 268, 772–778.

Ralevic, V., Kendall, D.A., Zygmunt, P.M., Movahed, P., Högestätt, E.D., 2000. Vanilloid receptors on capsaicin-sensitive sensory nerves mediate relaxation to methanandamide in the rat isolated mesenteric arterial bed. Br. J. Pharmacol. 130, 1483–1488.

Randall, M.D., Kendall, D.A., 1998. Endocannabinoids: a new class of vasoactive substances. Trends Pharmacol. Sci. 19, 55–58.

Randall, M.D., Alexander, S.P.H., Bennett, T., Boyd, E.A., Fry, J.R., Gardiner, S.M., Kemp, P.A., McCulloch, A.I., Kendall, D.A., 1996. An endogenous cannabinoid as an endothelium-derived vasorelaxant. Biochem. Biophys. Res. Commun. 229, 114–120.

Rinaldi-Carmona, M., Barth, F., Héaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, S., Maruani, J., Néliat, G., Caput, D., Ferrara, P., Soubrié, P., Brelière, J.C., Le Fur, G., 1994. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett. 350, 240–244.

Ross, R.A., Coutts, A.A., McFarlane, S.M., Anavi-Goffer, S., Irving, A.J., Pertwee, R.G., MacEwan, D.J., Scott, R.H., 2001. Actions of cannabinoid receptor ligands on rat cultured sensory neurones: implications for antinociception. Neuropharmacology 40, 221–232.

Rubino, A., Ralevic, V., Burnstock, G., 1993. The P_1-purinoceptors that mediate the prejunctional inhibitory effect of adenosine on capsaicin-sensitive nonadrenergic noncholinergic neurotransmission in the rat mesenteric arterial bed are of the A_1 subtype. J. Pharmacol. Exp. Ther. 267, 1100–1104.

Sullivan, J.M., 1999. Mechanisms of cannabinoid receptor-mediated inhibition of synaptic transmission in cultured hippocampal pyramidal neurons. J. Neurophysiol. 82, 1286–1294.

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.

Walker, J.M., Hohmann, A.G., Martin, W.J., Strangman, N.M., Huang, S.M., Tsou, K., 1999. The neurobiology of cannabinoid analgesia. Life Sci. 65, 665–673.

White, R., Hiley, C.R., 1998a. The actions of some cannabinoid receptor ligands in the rat isolated mesenteric artery. Br. J. Pharmacol. 125, 533–541.

White, R., Hiley, C.R., 1998b. The actions of the cannabinoid receptor antagonist, SR141716A, in the rat isolated mesenteric artery. Br. J. Pharmacol. 125, 689–696.

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 the vasodilator action of anandamide. Nature 400, 452–457.