Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 507 (2005) 211–221

www.elsevier.com/locate/ejphar

Vascular effects of Δ^9-tetrahydrocannabinol (THC), anandamide and *N*-arachidonoyldopamine (NADA) in the rat isolated aorta

Saoirse E. O'Sullivan*, David A. Kendall, Michael D. Randall

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

Received 7 October 2004; received in revised form 23 November 2004; accepted 26 November 2004
Available online 6 January 2005

Abstract

The vascular effects of cannabinoids have been compared in the rat isolated aorta. Δ^9-Tetrahydrocannabinol (THC), anandamide and *N*-arachidonoyl-dopamine (NADA) all caused vasorelaxation to similar degrees in pre-constricted aortae. Vasorelaxation to THC was inhibited by in vivo pre-treatment with pertussis toxin (10 μg/kg) or with the synthetic cannabinoid CP55,940 (((−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol), acutely or chronically), exposure to capsaicin in vitro (10 μM for 1 h), and de-endothelialisation. Vasorelaxation to anandamide was only inhibited by pertussis toxin and chronic CP55,940 pre-treatment (0.4 mg/kg for 11 days). Vasorelaxation to NADA was inhibited by pertussis toxin and chronic CP55,940 pre-treatment, and by de-endothelialisation. The vasorelaxant effects of the cannabinoids were not inhibited by cannabinoid CB_1 receptor antagonism; however, vasorelaxation to both CP55,940 and THC was inhibited by cannabinoid CB_2 receptor antagonism. Vasorelaxation to all cannabinoids was enhanced in the presence of indomethacin (10 μM). THC also caused vasoconstriction of the aorta while anandamide, NADA, CP55,940 and WIN 55,212-2 (*R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4benzoxazin-yl]-(1-naphthalenyl)methanone mesylate) did not. The vasoconstrictor effects of THC were inhibited by in vivo pre-treatment with pertussis toxin or CP55,940, acute exposure to CP55,940, cannabinoid CB_1 receptor antagonism and cyclooxygenase inhibition. These results demonstrate the opposing vascular effects of cannabinoids in the rat aorta, and although vasorelaxation to each of the cannabinoids is of similar magnitude, it is mediated through different pathways. This gives further indication of the different vascular actions of cannabinoid compounds.

Keywords: Cannabinoids; Anandamide; THC (Tetrahydrocannabinol); Aorta; Endothelium; Vanilloid

1. Introduction

The vasorelaxant effects of the first identified endocannabinoid, anandamide, have been widely studied, and the majority of evidence identifies sensory nerves, the endothelium, modulation of ion channels and presynaptic inhibition of sympathetic tone as factors contributing to its effects. Interestingly, the participation of the first identified cannabinoid receptor (CB_1) remains controversial (for review, see Randall et al., 2004), and there is much evidence that points to novel receptor sites for anandamide (Wiley and Martin, 2002; Pertwee, 2004).

Other endocannabinoids have also been shown to cause vasorelaxation of pre-constricted arterial preparations. *N*-arachidonoyl-dopamine (NADA, Bisogno et al., 2000) causes both endothelium-dependent and sensory nerve-mediated vasorelaxation of mesenteric arteries (O'Sullivan et al., 2004a), 2-arachidonoylglycerol (2-AG) causes endothelium-independent vasorelaxation mediated by both cannabinoid CB_1 and CB_2 receptors in mesenteric arteries (Kagota et al., 2001), and virodhamine causes endothelium-dependent relaxation that is not mediated by either cannabinoid (CB_1 or CB_2) or vanilloid receptors, also in mesenteric vessels (Ho and Hiley, 2003b). Of the plant cannabinoid compounds, Δ^9-tetrahydrocannabinol (THC)

* Corresponding author. Tel.: +44 115 9709484; fax: +44 115 9709259.
E-mail address: saoirse.o'sullivan@nottingham.ac.uk (S.E. O'Sullivan).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.11.056

has been shown to cause indomethacin-sensitive relaxation in rabbit cerebral vessels (Ellis et al., 1995), cannabinoid CB_1 receptor-mediated relaxation in rabbit mesenteric arteries (Fleming et al., 1999), and sensory nerve-mediated relaxation, also in rat mesenteric vessels (Zygmunt et al., 2002). The synthetic cannabinoid CB_1/CB_2 receptor agonist, HU-210 (*R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4benzoxazin-yl]-(1-naphthalenyl)methanone mesylate) is reported to cause cannabinoid CB_1-mediated vasorelaxation in isolated mesenteric arteries (White and Hiley, 1998; Fleming et al., 1999). However, WIN55,212 (*R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4benzoxazinyl]-(1-naphthalenyl)methanone mesylate), another synthetic cannabinoid CB_1 receptor agonist, produces vasorelaxation of rat mesenteric arteries that is not mediated by stimulation of either cannabinoid CB_1, CB_2 or vanilloid receptors (White and Hiley, 1998; Ho and Hiley, 2003a). Similarly, the cannabidiol analogue, abnormal cannabidiol, elicits endothelium-dependent non-CB_1/CB_2/vanilloid receptor-mediated relaxation of rat mesenteric vessels (Járai et al., 1999; Ho and Hiley, 2003a; Offertáler et al., 2003). Collectively, these data indicate that the mechanisms of the vascular effects of cannabinoids differ despite similar endpoints. Whether these differences are due to methodological, species or vascular bed variations remains to be established (see Randall et al., 2004).

We have recently reported that there are differences in the mechanisms of action of cannabinoids between third-order branches of the superior mesenteric artery, and the superior mesenteric artery, which is probably due to non-expression and/or functioning of the novel endothelial cannabinoid receptor in the larger artery (O'Sullivan et al., 2004a,b). Vanheel and Van de Voorde (2001) also reported that anandamide produced capsazepine-sensitive (i.e. vanilloid receptor-mediated) hyperpolarisations of third-order branches of the mesenteric artery, but not of the superior mesenteric artery. Similarly, a study by Andersson et al. (2002) showed in guinea pigs that, while anandamide is a full agonist at the vanilloid receptor mesenteric arteries, it is a partial agonist of the vanilloid receptor in main bronchi, and the authors attributed this to a difference in the efficacy of the vanilloid receptor between different tissues. This suggests that the vascular effects of cannabinoids may be dependent on the expression of receptor(s) or post-receptor pathways coupled to receptors in a given artery, or in the efficacy of each cannabinoid at the various receptors. This may account for some of the conflicting findings with regard to mechanisms of action for cannabinoid compounds.

The aim of this investigation was therefore to resolve some of the above issues by comparing the mechanisms of action of three cannabinoid compounds in the same artery. We chose the aorta, as to date, there is little published data on the effects of cannabinoids in this artery, and it was of interest to see how the aorta would compare to our previous findings in another conduit artery, the superior mesenteric artery (O'Sullivan et al., 2004a,b). We compared the vasorelaxant actions of THC with the endocannabinoids anandamide and NADA, with focus on the roles of sensory nerves (the vanilloid receptor), the cannabinoid CB_1 receptor, and endothelium in vasorelaxation to each cannabinoid.

2. Methods

2.1. Preparation of the rat aorta

Male Wistar rats (250–350 g) were stunned by a blow to the cranium and killed by cervical dislocation. The aorta was removed rapidly and placed into cold placed Krebs–Henseleit's buffer (composition, mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2, D-glucose 10). The aortae were dissected free of adherent connective and adipose tissue and cut into rings 5–8 mm long. The rings were placed in 50 ml organ baths containing Krebs–Henseleit's buffer and the solution was maintained at 37 °C and gassed with 5% CO_2 and 95% O_2. The rings were mounted between two stainless steel hooks and attached by thread to an isometric force displacement transducer (LETICA 210, Barcelona, Spain). Tension was measured and was recorded on a MacLab 4e recording system (ADInstruments, Oxfordshire, UK). Vessels were stretched to an optimal passive tension of 1 g and allowed to equilibrate (Tep-areenan et al., 2003). The contractile integrity of each vessel was tested by its ability to contract in the presence of 60 mM KCl by at least 0.5 g (typically 0.65 g contraction to KCl).

2.2. Treatment protocols

Pertussis toxin prevents guanine diphosphate release from the subunits of $G_{(i/o)}$-protein-coupled receptors, locking them in an inactive state. To assess potential $G_{(i/o)}$-protein-coupled receptor involvement in the vascular effects of cannabinoids, some animals were therefore injected intraperitoneally with pertussis toxin (one 10 μg/kg injection 3 days before the day of the experiment, Ninomiya et al., 2002). Both the cannabinoid CB_1 and CB_2 receptors are $G_{(i/o)}$-protein-coupled receptors (Pertwee, 2004). To induce cannabinoid tolerance, some animals were chronically treated with the synthetic cannabinoid receptor agonist CP55,940 (((−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol) at a dose of 0.4 mg/kg per day intraperitoneally at a volume of 1 ml/kg for 11 days (Fan et al., 1994). Vehicle controls animals were injected with ethanol/cremaphor/saline (1:1:18) at the same volume. This treatment protocol did not affect the contractile responses of aortae (vehicle 1.04±0.07 g increase tension, n=18; CP55,940 treated 0.96±0.04 g increase tension, n=19). The acute effects of CP55,940

were also assessed by incubating vessels with CP55,940 (1 μM, 1 h) and then the vasorelaxant effects of THC, NADA or anandamide were assessed as cumulative concentration–response curves.

2.3. Experimental protocol

For the relaxation studies, viable vessels were contracted with U46619, a thromboxane mimetic, to increase tension by at least 0.5 g. The average tension achieved was approximately 1 g. Once a stable contraction was achieved, the vasorelaxant effects of THC, NADA or anandamide were assessed as cumulative concentration–response curves. The steady-state response to the cannabinoids was recorded at each concentration and expressed as a percentage relaxation of the imposed U46619 contraction.

To assess whether cannabinoids act via $G_{(i/o)}$-protein-coupled receptors, some experiments were performed in aortae obtained from animals pre-treated with pertussis toxin. In some experiments, aortae were obtained from animals chronically pre-treated with a cannabinoid compound, CP55,940. Chronic cannabinoid treatment is thought to de-sensitise cannabinoid receptors, and this approach was traditionally used to establish whether the actions of cannabinoids were receptor-mediated. To assess the involvement of the cannabinoid receptors, either SR141716A (*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide, 1 μM, a cannabinoid CB_1 receptor antagonist, Rinaldi-Carmona et al., 1994), AM251 (*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide, 1 μM, a cannabinoid CB_1 receptor antagonist, Gatley et al., 1996) or SR144528 (*N*-[(1*S*)-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)pyrazole-3 carboxamide, 1 μM, a cannabinoid CB_2 receptor antagonist, Rinaldi-carmona et al., 1998) were added to the preparations 10 min before contraction. To assess the involvement of vanilloid receptors, some vessels were incubated for 1 h with the vanilloid agonist capsaicin (10 μM) to allow depletion of neurotransmitters from sensory nerves, and this was followed by a 20-min washout (Zygmunt et al., 1999). In some preparations, the endothelium was removed by abrasion with a hyperdermic needle. Preparations were considered denuded when relaxation to 10 μM carbachol, an endothelium-dependent vasorelaxant, was less than 20% of the imposed tone. The potential involvement of vasodilator prostanoids was assessed using the cyclooxygenase inhibitor indomethacin (10 μM), which was present in the buffer throughout the experiment.

In the contraction studies, once stable baseline tension was established, NADA, anandamide, or THC were added to the organ bath in a cumulative manner. To characterise the contractile response to THC, in some experiments, aortae were obtained from animals pre-treated with pertussis toxin or CP55,940. In some experiments, the cannabinoid CB_1 receptor antagonists, SR141716A (1 μM) or AM251 (1 μM) were added to the buffer 10 min before construction of the concentration–response curve. Additionally, the potential involvement of vasoconstrictor prostanoids was assessed using the cyclooxygenase inhibitors, indomethacin (10 μM) and flurbiprofen (10 μM). The contractile effects of the cannabinoid CB_1 receptor agonist WIN55,212-2 (*R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-*de*]-1,4benzoxazin-yl]-(1-naphthalenyl)methanone mesylate) and the cannabinoid CB_1/CB_2 receptor agonist CP55,940 were also assessed in the aorta. To evaluate whether either of these compounds antagonised the contractile effects of THC, 30 μM THC was added to the organ bath at the end of the concentration–response curve to both cannabinoid CB_1 agonists.

2.4. Statistical analysis

The concentration of vasorelaxant giving the half-maximal response (EC_{50}) was obtained from the concentration–response curve fitted to a sigmoidal logistic equation with the minimum vasorelaxation set to zero using the GraphPad Prism package (Tep-areenan et al., 2003). Maximal and pEC_{50} responses are expressed as

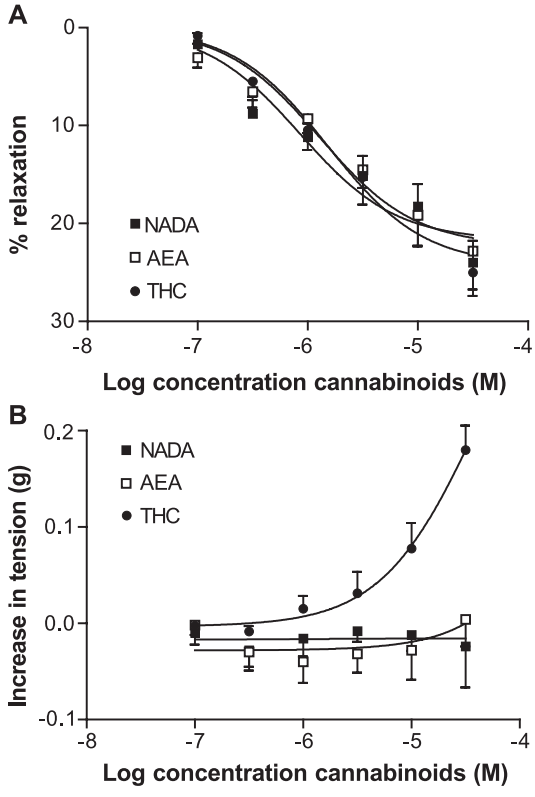

Fig. 1. The vascular effects of cannabinoids on pre-constricted rat aortae (A) and on baseline tone (B). Data are shown as mean±S.E.M.

mean±S.E.M. The number of animals in each group is represented by n. Data were compared by analysis of variance (ANOVA) with statistical significance between manipulations and controls determined by Dunnett's post hoc test. Data obtained from CP55,940 treated animals were compared with vehicle treated animals by Student's t-test.

2.5. Drugs and chemicals

All drugs were supplied by Sigma Chemical (Dorset, UK) except where stated. Anandamide, NADA, WIN55,212-2, CP55,940 and AM251 were obtained from Tocris (Avonmouth, UK). SR141716A was supplied by Research Biochemicals International (Natick, MA) as part of the Chemical Synthesis Programme of the National Institute of Mental Health contract (NOIMH3003). NADA, anandamide, CP55,940, capsaicin, and SR141716A were dissolved in ethanol to 10 mM with further dilutions made in distilled water. AM251 was initially dissolved in dimethyl sulfoxide (DMSO) to 10 mM, with further dilutions in distilled water. Flurbiprofen was dissolved in distilled water and indomethacin was dissolved in ethanol.

3. Results

All cannabinoids tested caused concentration-dependent relaxation of similar magnitude and with similar potency

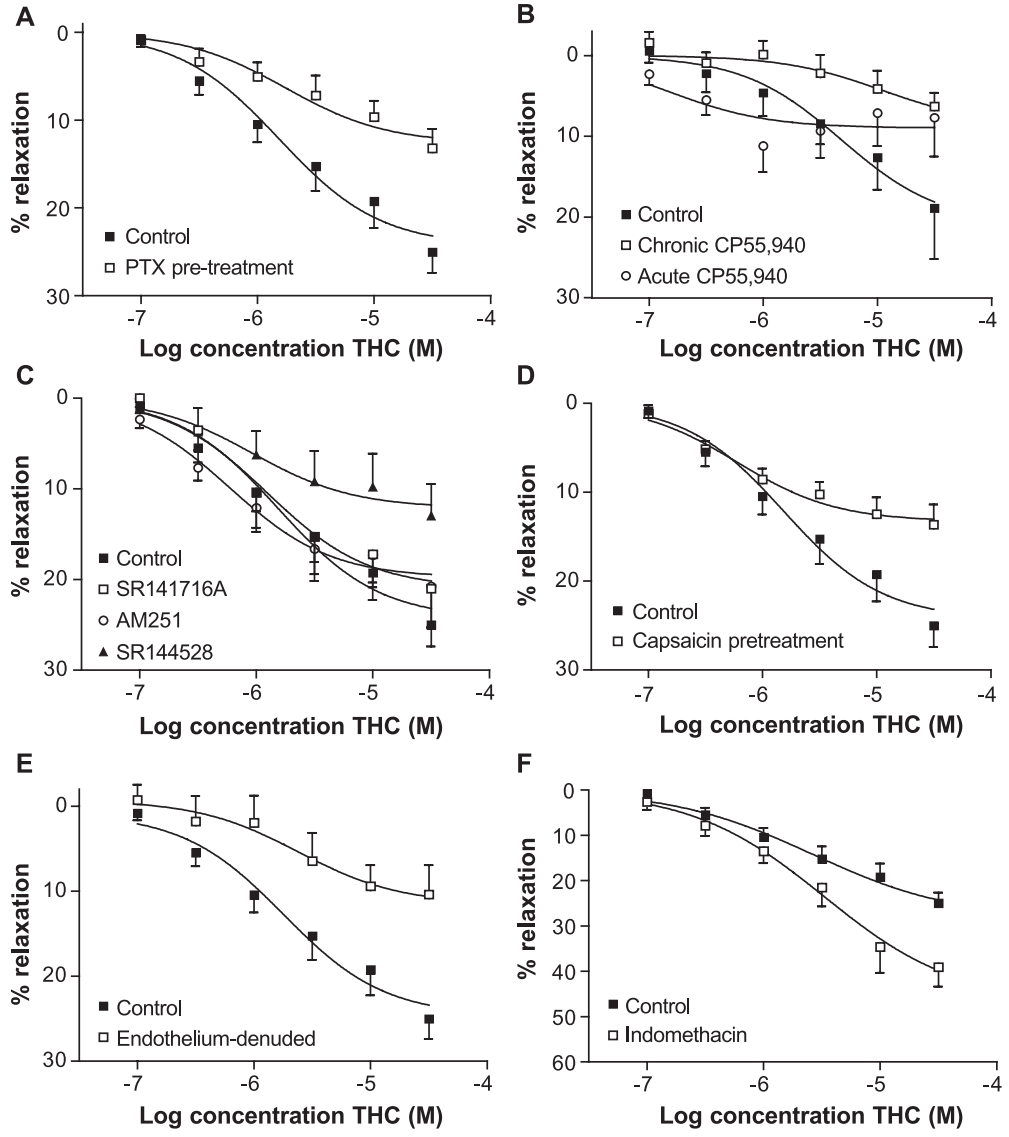

Fig. 2. The effects of pertussis toxin treatment (A, 10 μg/kg), acute (1 μM, 1 h) and chronic (0.4 mg/kg/day for 11 days) cannabinoid treatment with CP55,940 (B), the cannabinoid receptor antagonists SR141716A, AM251 and SR144528 (C, all 1 μM), capsaicin treatment (D, 10 μM for 1 h), endothelium denudation (E) and cyclooxygenase inhibition by indomethacin (10 μM) on vasorelaxation to Δ^9-tetrahydrocannabinol (THC) in the rat aorta. Data are shown as mean±S.E.M.

in pre-constricted rat isolated aortae (see Fig. 1A); anandamide (pEC_{50}=5.90±0.17; response at 30 μM (R_{max})=22.4±2.1% relaxation, n=10), NADA (pEC_{50}=6.07±0.13; R_{max}=21.8±1.5% relaxation, n=10) and THC (pEC_{50}=5.81±0.15; R_{max}=24.3±2.1% relaxation, n=10). THC also caused significant contraction in the aorta in the absence of a contractile agent (response at 30 μM=0.18±0.03 g tension, n=8). Neither endocannabinoid caused a significant change in baseline tension under these conditions (response at 30 μM; anandamide 0.004±0.03 g tension, n=5; NADA=−0.024±0.04 g change in tension, n=5, Fig. 1B).

3.1. Vasorelaxation to THC

Precontracted aortae from animals pre-treated with pertussis toxin showed a significantly reduced vasorelaxant response to THC (control R_{max}=24.3±2.1% relaxation, n=10; pertussis toxin treated R_{max}=12.7±1.7% relaxation, P<0.01, n=10, Fig. 2A). Aortae from animals pre-treated either acutely (1 μM, 1 h) or chronically with the cannabinoid CP55,940 significantly reduced the vasorelaxant response to THC compared with vehicle-injected animals (vehicle, R_{max}=20.9±5.2% relaxation, n=6; acute CP55, 940 R_{max}=8.97±1.9% relaxation, n=7 P<0.05; chronic

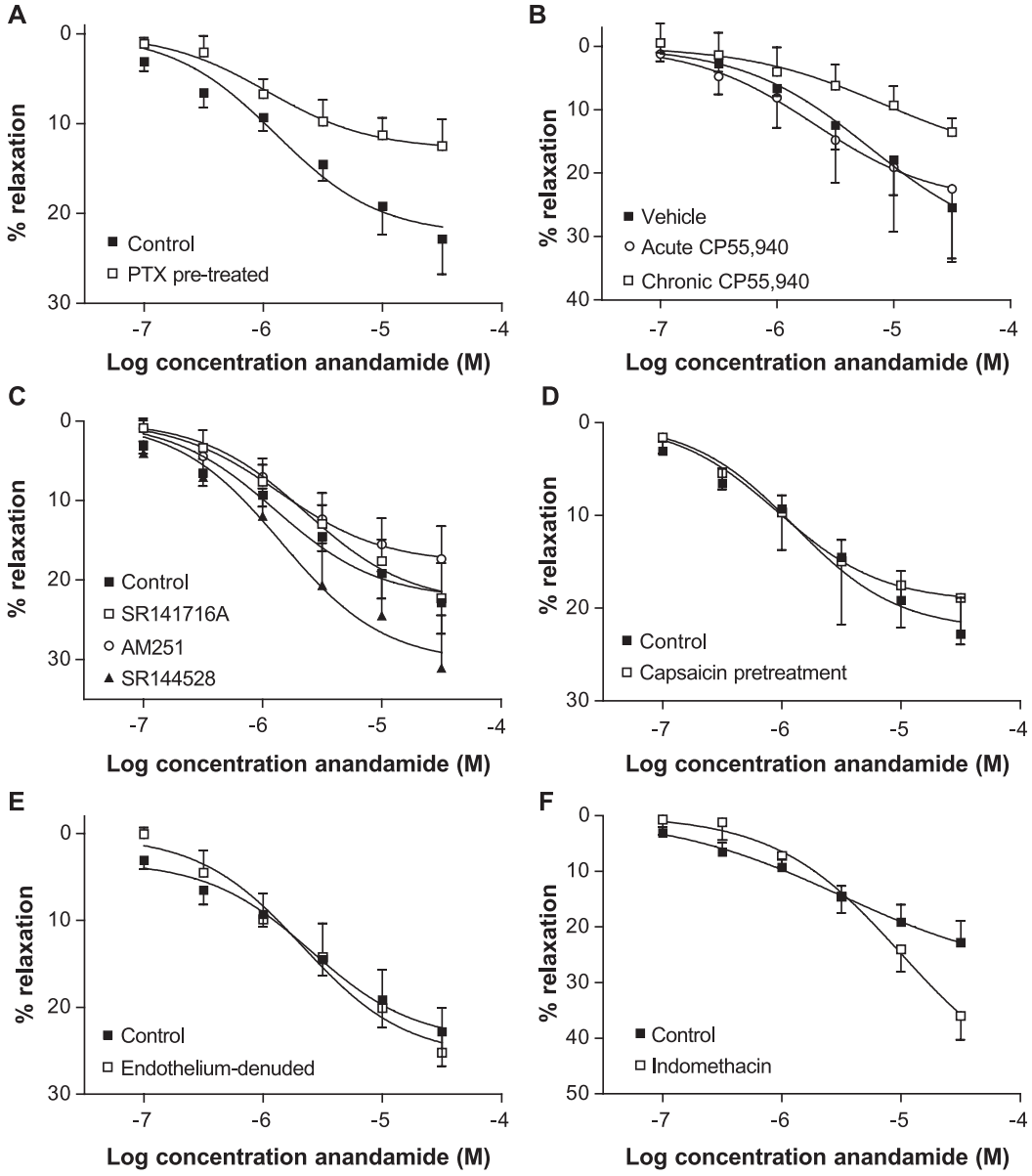

Fig. 3. The effects of pertussis toxin treatment (A, 10 μg/kg), acute (1 μM, 1 h) and chronic (0.4 mg/kg/day for 11 days) cannabinoid treatment with CP55,940 (B), the cannabinoid receptor antagonists SR141716A, AM251 and SR144528 (C, all 1 μM), capsaicin treatment (D, 10 μM for 1 h), endothelium denudation (E) and cyclooxygenase inhibition by indomethacin (10 μM) on vasorelaxation to anandamide in the rat aorta. Data are shown as mean±S.E.M.

CP55,940, R_{max}=8.6±4.7% relaxation, n=6, P<0.05, Fig. 2B). Vasorelaxation to THC was unaffected by either cannabinoid CB_1 receptor antagonist (SR141716A=R_{max} 21.0±2.8% relaxation, n=9; AM251=R_{max} 19.8±2.2% relaxation, n=10, Fig. 2C). Vasorelaxation to THC was significantly inhibited after treatment with the vanilloid receptor agonist capsaicin (R_{max}=13.8±1.1% relaxation, n=9, P<0.01, Fig. 2D), and after removal of the endothelium (R_{max}=11.5±2.7% relaxation, n=7, P<0.01, Fig. 2E). In the presence of indomethacin, vasorelaxation to THC was enhanced in the rat aorta (R_{max}=41.6±3.8% relaxation, n=6, P<0.01, Fig. 2F, note different scale).

3.2. Vasorelaxation to anandamide

Aortae from animals pre-treated with pertussis toxin showed a significantly reduced vasorelaxant response to anandamide (control R_{max}=22.4±2.1% relaxation, n=10; pertussis toxin treated R_{max}=12.8±1.7% relaxation, P<0.05, n=8, Fig. 3A). Aortae from animals pre-treated with CP55,940 also had a reduced vasorelaxant response to anandamide compared with vehicle-injected animals (vehicle, R_{max}=27.5±5.4% relaxation, n=5; CP55,940 treated, R_{max}=14.7±4.6% relaxation, n=6, P<0.01, Fig. 3B). Acute treatment with CP55,940 (1 μM, 1 h) did not

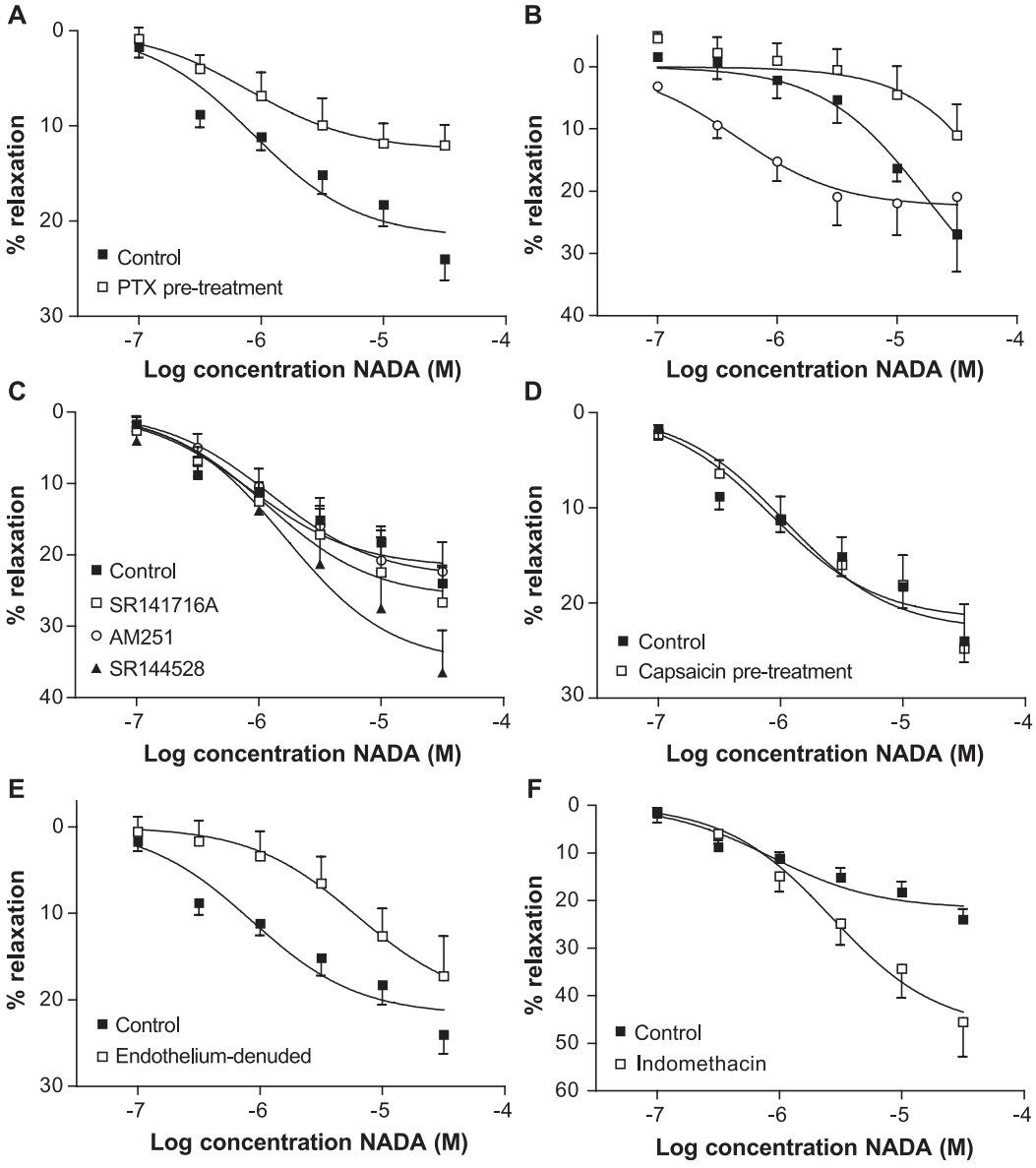

Fig. 4. The effects of pertussis toxin treatment (A, 10 μg/kg), acute (1 μM, 1 h) and chronic (0.4 mg/kg/day for 11 days) cannabinoid treatment with CP55,940 (B), the cannabinoid receptor antagonists SR141716A, AM251 and SR144528 (C, all 1 μM), capsaicin treatment (D, 10 μM for 1 h), endothelium denudation (E) and cyclooxygenase inhibition by indomethacin (10 μM) on vasorelaxation to *N*-arachidonoyl-dopamine (NADA) in the rat aorta. Data are shown as mean±S.E.M.

affect the vasorelaxant responses to anandamide (acute CP55,940, R_{max}=24.6±14.0% relaxation, n=7, Fig. 3B). Vasorelaxation to anandamide was not affected by either cannabinoid CB_1 receptor antagonist (SR141716A R_{max}=23.0±2.8% relaxation, n=7; AM251 R_{max}=17.9±2.5% relaxation, n=9, Fig. 3C), by treatment with the vanilloid receptor agonist, capsaicin (R_{max}=19.3±3.4% relaxation, n=7, Fig. 3D), or after removal of the endothelium (R_{max}=25.6±3.6% relaxation, n=7, Fig. 3E). In the presence of indomethacin, relaxation to the highest concentration of anandamide was enhanced in the rat aorta (R_{max}=42.5±5.6% relaxation, n=6, P<0.01, Fig. 3F, note different scale).

3.3. Vasorelaxation to NADA

Aortae from animals pre-treated with pertussis toxin showed a significantly reduced vasorelaxant response to NADA (control R_{max}=21.8±1.5% relaxation, n=10; pertussis toxin treated R_{max}=12.5±1.6% relaxation, n=6, P<0.05, Fig. 4A). Pre-treatment with CP55,940 significantly reduced the vasorelaxant response to NADA compared with vehicle

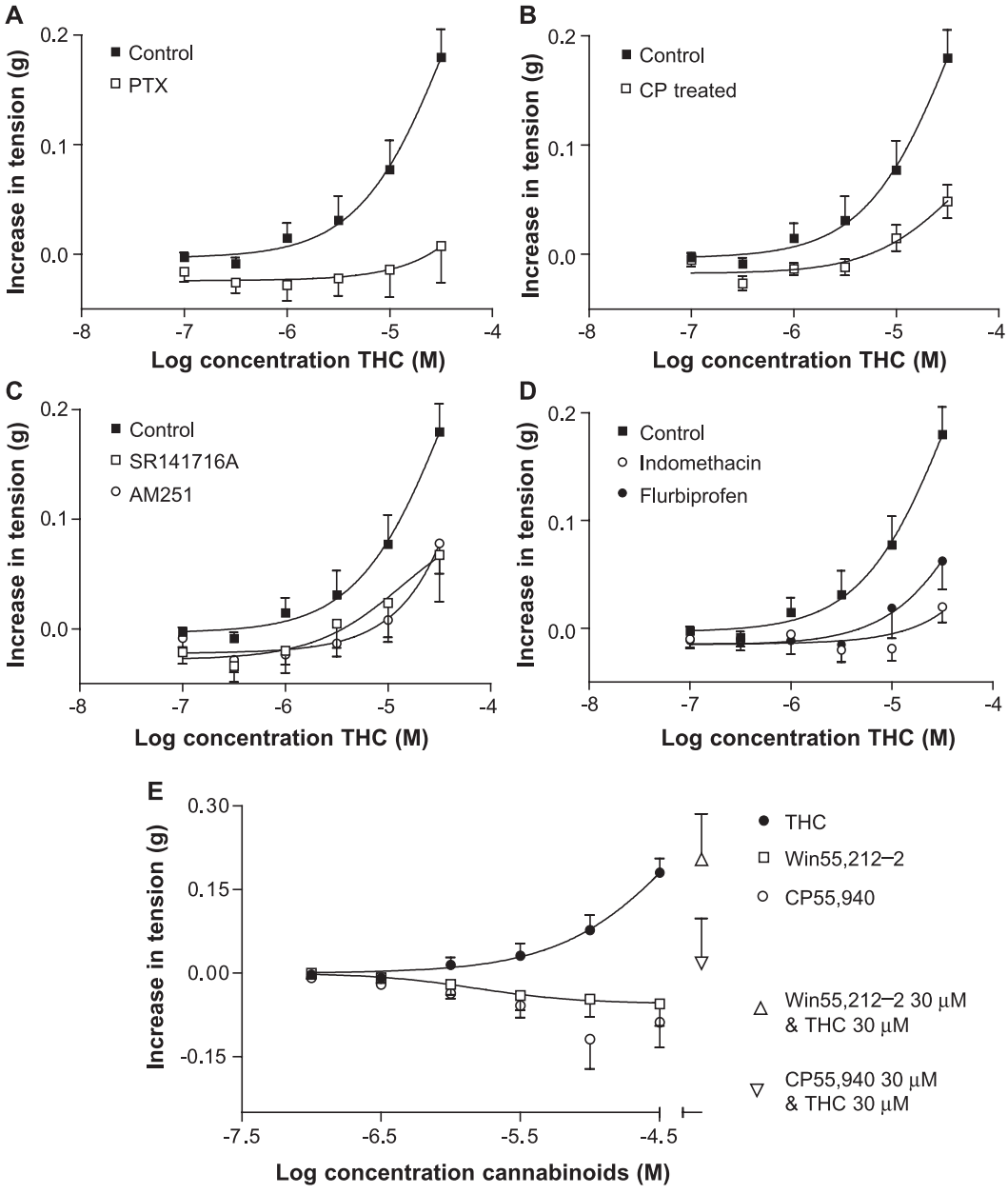

Fig. 5. The effects of pertussis toxin treatment (A, 10 μg/kg) and chronic (0.4 mg/kg/day for 11 days) cannabinoid treatment with CP55,940 (B), the cannabinoid CB_1 receptor antagonists SR141716A and AM251 (C, both 1 μM), and cyclooxygenase inhibition by indomethacin (10 μM) or flurbiprofen (10 μM) on the vasoconstrictor effects of Δ^9-tetrahydrocannabinol (THC) in the rat aorta. (E) The effects of CP55,940 and WIN55,212-2 on the non-precontracted aorta, and on the vasoconstrictor effects of THC. Data are shown as mean±S.E.M.

injected animals (vehicle, R_{max}=39.1±11.3% relaxation, n=6; CP55,940 treated, R_{max}=17.8±13.3% relaxation, n=6, P<0.05, Fig. 4B). However, after acute exposure to CP55,940, the vasorelaxant potency of NADA was enhanced (vehicle, pEC_{50}=5.0±0.32, n=6; acute CP55,940 pEC_{50}=6.37±0.23, n=7, P<0.05, Fig. 4B). Vasorelaxation to NADA was not affected by either cannabinoid CB_1 receptor antagonist (SR141716A R_{max}=26.0±3.0% relaxation, n=7; AM251 R_{max}=23.1±2.7% relaxation, n=7, Fig. 4C), or by treatment with the vanilloid receptor agonist, capsaicin (R_{max}=22.9±2.4% relaxation, n=7, Fig. 4D). After removal of the endothelium, the potency of NADA was reduced in the rat aorta without a change in the vasorelaxant response to the highest concentration of NADA tested (control pEC_{50}=6.07±0.13, n=10; endothelium-denuded=5.21±0.32, n=6, P<0.05, Fig. 4E). In the presence of indomethacin, vasorelaxation to NADA was enhanced (R_{max}=47.1±5.1% relaxation, n=6, P<0.01, Fig. 4F).

3.4. Vasoconstriction to THC

In the absence of pharmacologically induced tone, aortae from animals pre-treated with pertussis toxin did not contract to THC (R_{max}=0.01±0.03 g tension, P<0.01, Fig. 5A). Aortae from animals treated chronically with CP55,940 did not show a contractile response to THC (R_{max}=0.05±0.02 g tension, P<0.05, n=6, Fig. 5B). Pre-incubation of aortic rings with the cannabinoid CB_1 receptor antagonists SR141716A (1 μM) or AM251 (1 μM) caused a reduction of the contractile response to THC (R_{max}=0.06±0.04 g tension, P<0.05, n=9, Fig. 5C). In the presence of 10 μM indomethacin, aortae did not contract to THC (R_{max}=0.02±0.01 g tension, P<0.01, n=9, Fig. 5D). Similarly, in the presence of flurbiprofen, the contractile response to THC in the aorta was significantly reduced (10 μM, R_{max}=0.06±0.03 g tension, P<0.05, n=8, Fig. 5D).

The cannabinoid CB_1 receptor agonist WIN55,212-2 was not found to cause vasoconstriction of the aorta (response at 30 μM=−0.05±0.04 g tension, n=6, Fig. 5E). The addition of 30 μM THC to the organ bath at the end of the concentration response to WIN55,212-2 resulted in contraction to THC (R_{max} 0.20±0.08 g tension, Fig. 5E). The cannabinoid CB_1 receptor agonist CP55,940 also did not cause significant vasoconstriction of the aorta (response at 30 μM=−0.09±0.04 g tension, n=5, Fig. 5E). However, following the addition of 30 μM THC, the contractile response to THC was diminished (0.02±0.08 g tension, n=5, Fig. 5E).

3.5. Cannabinoid CB_2 receptors in the aorta

CP55,940 caused significant vasorelaxation of the aorta which was more efficacious than any of the other cannabinoids tested (pEC_{50}=5.27±0.13; response at 30 μM=96.3±9.4% relaxation, n=8, Fig. 6A). The cannabinoid CB_1 receptor antagonist SR141716A had no effect on vasorelaxation to CP55,940 (pEC_{50}=5.24±0.18, n=6), but the cannabinoid CB_2 receptor antagonist SR144528 caused a significant rightward shift in the dose–response curve to CP55,940 (pEC_{50}=4.63±0.24, P<0.05, n=6, Fig. 6A). THC, but not anandamide or NADA, was also reduced in the presence of the cannabinoid CB_2 receptor antagonist SR144528 (see Figs. 2, 3 and 4C).

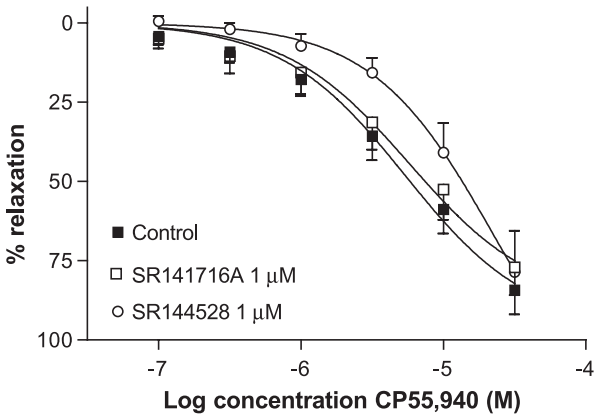

Fig. 6. The effects of cannabinoid CB_1 (SR141716A, 1 μM) and CB_2 receptor antagonism (SR144528, 1 μM) on vasorelaxation to CP55,940. Data are shown as mean±S.E.M.

4. Discussion

This study has characterised for the first time the vascular effects of a number of cannabinoids in the rat aorta. We have shown that while all cannabinoids cause vasorelaxation of pre-constricted aortae, there is not a common mechanism of action; THC acts by a $G_{(i/o)}$-protein-linked site, sensory nerves, and an endothelial site; anandamide acts at a $G_{(i/o)}$-protein-linked site only; and NADA acts at a $G_{(i/o)}$-protein-linked site and the endothelium. Additionally, only THC acts as a contractile agent in non-precontracted arteries. Importantly, our data also show that prolonged exposure to a synthetic cannabinoid is accompanied by a reduction in the vascular effects of both endogenous and exogenous cannabinoids.

The present study found that each cannabinoid caused approximately 20–25% relaxation of precontracted aortae. To date, the vasorelaxant effects of cannabinoids in the aorta have only been reported by Mukhopadhyay et al. (2002), who found that anandamide caused a 60% endothelium-dependent relaxation of the rabbit aorta. This considerable difference in the maximal effect of anandamide may be due to a species difference in sensitivity to anandamide, as other rabbit arteries have also been shown to have greater responses to anandamide compared with the rat (Fleming et al., 1999; O'Sullivan et al., 2004b). In small resistance mesenteric rat arteries, we have shown that cannabinoids cause almost complete relaxation, but in the superior mesenteric artery, anandamide and NADA only cause about

30% relaxation (O'Sullivan et al., 2004a,b). Thus, there appears to be a decrease in the maximal effects of cannabinoids as vessels increase in size. It is not clear from our data whether this reduced sensitivity to cannabinoids is due to differences in receptor expression, or is purely a function of increased smooth muscle mass.

None of the cannabinoids tested were sensitive to either of the cannabinoid CB_1 receptor antagonists, SR141716A or AM251, suggesting the cannabinoid CB_1 receptor may not play a role in vasorelaxation to cannabinoids. In support of this, it was not found that the cannabinoid CB_1 receptor agonist WIN55,212-2 had any vasorelaxant effect on the aorta by Mukhopadhyay et al. (2002). Also, Holland et al. (1999) showed in the rat carotid artery that cannabinoid CB_1 receptors were in fact coupled to inhibition of adenylyl cyclase, which would cause vasoconstriction, not relaxation. Furthermore, when we tested the vasorelaxant effects of the cannabinoid CB_1/CB_2 receptor agonist CP55,940 (Howlett et al., 2002), we found that it was sensitive only to cannabinoid CB_2 receptor antagonism and not CB_1 receptor antagonism, as was vasorelaxation to THC. Collectively, these data cast doubt on the role of cannabinoid CB_1 receptors in vasorelaxation to cannabinoids in the rat aorta.

We found that CP55,940 and THC, but not anandamide or NADA, were sensitive to the cannabinoid CB_2 receptor antagonist SR144528, suggesting a role for CB_2 receptors in vasorelaxation to some non-endogenous cannabinoids. This is of interest considering CP55,940 and THC have similar affinities for cannabinoid CB_1 and CB_2 receptors, while the endocannabinoids are selective for cannabinoid CB_1 over CB_2 (Howlett et al., 2002). It has been previously shown that cannabinoid CB_2 receptors are expressed in endothelial cell cultures (Zoratti et al., 2003), and our data may confirm the expression of cannabinoid CB_2 receptors in the rat aorta. However, there is previous evidence for other cannabinoid CB_2 receptor ligands causing non-CB_1/CB_2-sensitive vasorelaxation in mesenteric vessels (White and Hiley, 1998; Ho and Hiley, 2003a,b), and therefore it must also be considered that CP55,940 and THC are acting at a cannabinoid 'CB_2-like' receptor that is sensitive to SR144528 in the aorta.

The vasorelaxant responses to THC, anandamide and NADA were all inhibited by pertussis toxin treatment, indicating the involvement of a separate $G_{(i/o)}$-protein-linked receptor. A vascular $G_{(i/o)}$-protein-linked receptor is proposed to exist on the endothelium (Begg et al., 2003), which could account for the pertussis toxin-sensitive effects of THC and NADA, both of which were also inhibited by endothelial denudation. Indeed, we have previously demonstrated that approximately 50% of the relaxant effects of NADA in mesenteric resistance vessels is mediated through this receptor (O'Sullivan et al., 2004a). Furthermore, only the endothelial-dependent component of vasorelaxation to methanandamide in the rabbit aorta was pertussis toxin-sensitive (Mukhopadhyay et al., 2002), pointing at an endothelial receptor site of action. However, our results showed that vasorelaxation to anandamide was not only endothelium-independent but also pertussis toxin-sensitive, which could indicate the existence of another unidentified cannabinoid receptor located on the smooth muscle. Indeed, pharmacological evidence suggests that there may be many novel cannabinoid receptors in the vasculature that remain to be cloned (see Pertwee, 2004).

It is known that tolerance to cannabinoids is rapidly induced by chronic exposure to cannabinoids, although whether this is through a downregulation and/or desensitisation of receptors, or changes in intracellular signalling is not fully understood (Fan et al., 1996; Zhuang et al., 1998). A protocol of chronic cannabinoid treatments has been traditionally employed to identify cannabinoid receptor-mediated responses. Importantly, we have shown that the vascular effects, both vasoconstriction and vasorelaxation, of cannabinoids are reduced after the induction of tolerance with a synthetic cannabinoid receptor agonist, CP55,940. The cardiovascular effects of marijuana and THC also show rapid tolerance in humans (see Jones, 2002). We have also shown that cross-tolerance develops between cannabinoids. Similarly, Fan et al. (1994) reported cross-tolerance to THC by CP55,940, and vice versa, in markers of behavioural tolerance in mice. Interestingly, only vasorelaxation to THC was also inhibited by acute exposure to CP55,940, and based on our findings that both of these compounds are SR144528-sensitive, it might be supposed that this is through competition for the cannabinoid CB_2/'CB_2-like' receptor.

Zygmunt et al. (1999, 2002) have shown that the effects of anandamide and THC in rat mesenteric arteries are mediated through the stimulation of receptors on sensory nerves. To investigate whether sensory nerves play a role in relaxation to cannabinoids in the aorta, vessels were incubated with the vanilloid receptor agonist capsaicin to deplete sensory neurotransmitters. After this treatment, we found that only the vasorelaxant effects of THC were significantly reduced. Although both anandamide and NADA are capable of stimulating the vanilloid receptor and causing vasorelaxation in mesenteric vessels (Zygmunt et al., 1999; O'Sullivan et al., 2004a,b), it may be that their efficacy at the vanilloid receptor is less than that of THC in the aorta. Andersson et al. (2002) showed in guinea pigs that, while anandamide is a full agonist at the vanilloid receptor in mesenteric arteries, it is a partial agonist of this receptor in main bronchi. Only the metabolically stable analogue of anandamide, methanandamide, has been shown to relax the rabbit aorta in a capsaicin-sensitive manner (Mukhopadhyay et al., 2002), which might suggest that anandamide is metabolised before it reaches the vanilloid receptor.

We have shown, for the first time, vasoconstrictor effects of THC in the rat aorta, which were sensitive to pertussis toxin treatment, cannabinoid CB_1 receptor antagonists, and induction of cannabinoid tolerance. Holland et al. (1999) showed in the rat carotid artery that cannabinoid CB_1 receptors were coupled to inhibition of adenylyl cyclase, which would cause vasoconstriction instead of relaxation,

and this is a potential mechanism for the effects of THC. However, another more potent cannabinoid CB_1 receptor ligand, WIN55,212, or the cannabinoid CB_1/CB_2 receptor agonist CP55,940, did not cause vasoconstriction in the aorta. It may be that the inhibitory actions of the cannabinoid CB_1 receptor antagonists on THC-mediated vasoconstriction are through intrinsic vasorelaxant effects of SR141716A/AM251 counteracting the contractile effects of THC (White and Hiley, 1998). It has been recently reported that the endogenous cannabinoid 2-arachidonoyl glycerol (2-AG) causes contraction of the isolated rat aorta through metabolism to thromboxane receptor agonists (Stanke-Labesque et al., 2004), and we found that constriction to THC was also reduced after cyclooxygenase inhibition. It seems likely that vasoconstrictor prostanoids play a role in the vasoconstrictor response to THC. We have previously reported similar findings for THC in the superior mesenteric artery (O'Sullivan et al., 2004c). It is of note that vasorelaxation to all cannabinoids was enhanced in the presence of indomethacin, suggesting stimulation vasoconstrictor prostanoid pathways which simultaneously oppose the relaxant effects of the cannabinoids, perhaps in a regulatory fashion. The vascular outcome of stimulating multiple opposing pathways may depend on the prevailing conditions.

In summary, we have shown that contraction to THC in the rat aorta is mediated by a $G_{(i/o)}$-protein-linked receptor that can be de-sensitised by chronic cannabinoid agonist administration, and is inhibited after cyclooxygenase inhibition, indicating a role for vasoconstrictor prostanoids. Each cannabinoid tested produced similar relaxation in the aorta by different pathways, and also by a common unidentified pertussis toxin-sensitive receptor, and all vascular effects of cannabinoids were also reduced after chronic treatment with a synthetic cannabinoid compound. These data support the notion of additional vascular sites of action for cannabinoids, and highlight the different pharmacologies of cannabinoid compounds.

Acknowledgements

This study was funded by the British Heart Foundation (PG2001/150). The aortae from pre-treatment studies were a generous donation from Dr. Marnie Duncan.

References

Andersson, D.A., Adner, M., Hogestatt, E.D., Zygmunt, P.M., 2002. Mechanisms underlying tissue selectivity of anandamide and other vanilloid receptor agonists. Mol. Pharmacol. 62, 705–713.

Begg, M., Mo, F.M., Offertaler, L., Batkai, S., Pacher, P., Razdan, R.K., Lovinger, D.M., Kunos, G., 2003. G protein-coupled endothelial receptor for atypical cannabinoid ligands modulates a Ca^{2+}-dependent K^+ current. J. Biol. Chem. 278, 46188–46194.

Bisogno, T., Melck, D., Bobrov, M.Yu., Gretskaya, N.M., Bezuglov, V.V., De Petrocellis, L., Di Marzo, V., 2000. *N*-acyl-dopamines: novel synthetic CB(1) cannabinoid-receptor ligands and inhibitors of anandamide inactivation with cannabimimetic activity in vitro and in vivo. Biochem. J. 351, 817–824.

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.

Fan, F., Compton, D.R., Ward, S., Melvin, L., Martin, B.R., 1994. Development of cross-tolerance between delta 9-tetrahydrocannabinol, CP 55,940 and WIN 55,212. J. Pharmacol. Exp. Ther. 271, 1383–1390.

Fan, F., Tao, Q., Abood, M., Martin, B.R., 1996. Cannabinoid receptor down-regulation without alteration of the inhibitory effect of CP 55,940 on adenylyl cyclase in the cerebellum of CP 55,940-tolerant mice. Brain Res. 706, 13–20.

Fleming, I., Schermer, B., Popp, R., Busse, R., 1999. Inhibition of the production of endothelium-derived hyperpolarizing factor by cannabinoid receptor agonists. Br. J. Pharmacol. 126, 949–960.

Gatley, S.J., Gifford, A.N., Volkow, N.D., Lan, R., Makriyannis, A., 1996. 123I-labeled AM251: a radioiodinated ligand which binds in vivo to mouse brain cannabinoid CB1 receptors. Eur. J. Pharmacol. 307, 331–338.

Ho, W.S., Hiley, C.R., 2003a. Endothelium-independent relaxation to cannabinoids in rat-isolated mesenteric artery and role of Ca^{2+} influx. Br. J. Pharmacol. 139, 585–597.

Ho, W.S., Hiley, C.R., 2003b. Vasorelaxation to the novel endocannabinoid virodhamine. 13th International Cannabinoid Research Symposium, p. 89 (Abstract).

Holland, M., Challiss, R.A., Standen, N.B., Boyle, J.P., 1999. Cannabinoid CB_1 receptors fail to cause relaxation, but couple via Gi/Go to the inhibition of adenylyl cyclase in carotid artery smooth muscle. Br. J. Pharmacol. 128, 597–604.

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 CB1 or CB2 receptors. Proc. Natl. Acad. Sci. 96, 14136–14141.

Jones, R.T., 2002. Cardiovascular system effects of marijuana. J. Clin. Pharmacol. 42, 58S–63S.

Kagota, S., Yamaguchi, Y., Nakamura, K., Sugiura, T., Waku, K., Kunitomo, M., 2001. 2-Arachidonoylglycerol, a candidate of endothelium-derived hyperpolarizing factor. Eur. J. Pharmacol. 415, 233–238.

Mukhopadhyay, S., Chapnick, B.M., Howlett, A.C., 2002. Anandamide-induced vasorelaxation in rabbit aortic rings has two components: G protein dependent and independent. Am. J. Physiol. 282, H2046–H2054.

Ninomiya, H., Otani, H., Lu, K., Uchiyama, T., Kido, M., Imamura, H., 2002. Enhanced IPC by activation of pertussis toxin-sensitive and -insensitive G protein-coupled purinoceptors. Am. J. Physiol. 282, H1933–H1943.

Offertáler, L., Mo, F.M., Batkai, S., Liu, J., Begg, M., Razdan, R.K., Martin, B.R., Bukoski, R.D., Kunos, G., 2003. Selective ligands and cellular effectors of a G protein-coupled endothelial cannabinoid receptor. Mol. Pharmacol. 63, 699–705.

O'Sullivan, S.E., Kendall, D.A., Randall, M.D., 2004a. Characterisation of the vasorelaxant properties of the novel endocannabinoid *N*-arachidonoyl-dopamine (NADA). Br. J. Pharmacol. 141, 803–812.

O'Sullivan, S.E., Kendall, D.A., Randall, M.D., 2004b. Heterogeneity in the mechanisms of vasorelaxation to anandamide in resistance and conduit rat mesenteric arteries. Br. J. Pharmacol. 142, 435–442.

O'Sullivan, S.E., Kendall, D.A., Randall, M.D., 2004c. Vascular effects of Δ^9-tetrahydrocannabinol (THC) in isolated rat mesenteric arteries. Proceedings of the British Pharmacological Society at http://www.pa2online.org/Vol2Issue2abst013P.html.

Pertwee, R.G., 2004. Novel pharmacological targets for cannabinoids. Curr. Neuropharmacol. 2, 9–29 (review).

Randall, M.D., Kendall, D.A., O'Sullivan, S.E., 2004. The complexities of the cardiovascular actions of cannabinoids. Br. J. Pharmacol. 142, 20–26.

Rinaldi-Carmona, M., Barth, F., Heaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, S., Maruani, J., Neliat, G., Caput, D., et al., 1994. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett. 350, 240–244.

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., LeFur, G., 1998. SR144528, the first potent and selective antagonist of the CB_2 cannabinoid receptor. J. Pharmacol. Exp. Ther. 284, 644–650.

Stanke-Labesque, F., Mallaret, M., Lefebvre, B., Hardy, G., Caron, F., Bessard, G., 2004. 2-Arachidonoyl glycerol induces contraction of isolated rat aorta: role of cyclooxygenase-derived products. Cardiovasc. Res. 63, 155–160.

Tep-areenan, P., Kendall, D.A., Randall, M.D., 2003. Mechanisms of vasorelaxation to testosterone in the rat aorta. Eur. J. Pharmacol. 465, 125–132.

Vanheel, B., Van de Voorde, J., 2001. Regional differences in anandamide- and methanandamide-induced membrane potential changes in rat mesenteric arteries. J. Pharmacol. Exp. Ther. 296, 322–328.

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

Wiley, J.L., Martin, B.R., 2002. Cannabinoid pharmacology: implications for additional cannabinoid receptor subtypes. Chem. Phys. Lipids 121, 57–63.

Zhuang, S., Kittler, J., Grigorenko, E.V., Kirby, M.T., Sim, L.J., Hampson, R.E., Childers, S.R., Deadwyler, S.A., 1998. Effects of long-term exposure to delta9-THC on expression of cannabinoid receptor (CB1) mRNA in different rat brain regions. Brain Res. Mol. Brain Res. 62, 141–149.

Zoratti, C., Kipmen-Korgun, D., Osibow, K., Malli, R., Graier, W.F., 2003. Anandamide initiates Ca(2+) signaling via CB2 receptor linked to phospholipase C in calf pulmonary endothelial cells. Br. J. Pharmacol. 140, 1351–1362.

Zygmunt, P.M., Petersson, J., Andersson, D.A., Chuang, H.H., Sorgard, M., DI Marzo, V., Julius, D., Hogestatt, E.D., 1999. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400, 452–457.

Zygmunt, P.M., Andersson, D.A., Hogestatt, E.D., 2002. Delta 9-tetrahydrocannabinol and cannabinol activate capsaicin-sensitive sensory nerves via a CB1 and CB2 cannabinoid receptor-independent mechanism. J. Neurosci. 22, 4720–4727.