The in Vitro and in Vivo Cardiovascular Effects of Δ⁹-Tetrahydrocannabinol in Rats Made Hypertensive by Chronic Inhibition of Nitric-Oxide Synthase

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ABSTRACT
Evidence suggests that Δ⁹-tetrahydrocannabinol (THC) may have antihypertensive effects and that the vasodilator effect of endocannabinoids is enhanced in rats made hypertensive by chronic NO synthase inhibition. Therefore, the aims of the present study were to investigate whether the in vitro and in vivo cardiovascular responses to THC are altered by chronic NO synthase inhibition. Therefore, the aims of the present study were to investigate whether the in vitro and in vivo cardiovascular responses to THC are altered by chronic NO synthase inhibition. Therefore, the aims of the present study were to investigate whether the in vitro and in vivo cardiovascular responses to THC are altered by chronic NO synthase inhibition.

The first in vitro vascular study showed that THC was capable of relaxing rabbit cerebral arterioles (Ellis et al., 1995) through stimulation of prostaglandins. Subsequent research showed THC-mediated endothelium-independent vasorelaxation of the rabbit superior mesenteric artery (Fleming et al., 1999) and vasorelaxation of the rat hepatic and mesenteric arteries through actions on sensory nerves, although not through the archetypal TRPV1 receptor (Zygmunt et al., 2002). We have recently shown that additional mechanisms underlying vasorelaxation to THC in rat mesenteric vessels include activation of a G protein-coupled receptor, inhibition of calcium channels, and activation of potassium channels (O’Sullivan et al., 2005). THC-based medicines possess interesting therapeutic potential as antiemetics, appetite stimulants, and analgesics and in the treatment of multiple sclerosis, epilepsy, and glaucoma (Ben Amar, 2006). Given the vascular actions of THC (Ellis et al., 1995, Fleming et al., 1999; Zygmunt et al., 2002; O’Sullivan et al., 2005), it is of interest to investigate any therapeutic potential of THC in cardiovascular disease and/or any potential cardiovascular side effects when used...
for other conditions. Research in the 1970s found that chronic THC treatment caused larger and longer lasting hypotension in conscious spontaneously hypertensive rats (SHRs) than in normotensive rats (Kosersky, 1978) and that inhalation of THC caused a greater reduction in blood pressure in patients with glaucoma with high blood pressure than in normotensive patients (Crawford and Merritt, 1979). These early studies might indicate a potential antihypertensive effect of THC. The first aim of the present study was therefore to investigate the vascular responses to THC in isolated mesenteric vessels, as measured by myography (O'Sullivan et al., 2005), in rats made hypertensive by administration of the nitric-oxide synthase (NOS) inhibitor Nω-nitro-arginine methyl ester (l-NAME at 10 mg kg⁻¹ day⁻¹ for 4 weeks) (for review see Zatz and Baylis, 1998).

It has been shown previously that the vasorelaxant response to the endogenous cannabinoid anandamide is enhanced after chronic NOS inhibition (Mendizábal et al., 2001).

Despite the collective evidence of THC-mediated vasorelaxation in vitro, the mechanisms underlying any depressor effect of THC in vivo remain unclear. In anesthetized animals, a sustained hypotensive response to various cannabinoids agonists, including anandamide and THC, has been reported previously (Lake et al., 1997a,b; Malinowska et al., 2001; Wagner et al., 2001). This hypotensive effect can be antagonized by cannabinoid CB1 receptor antagonists (Lake et al., 1997a; Malinowska et al., 2001; Wagner et al., 2001), and it may involve peripheral prejunctional inhibition of noradrenaline release from postganglionic sympathetic axons (Varga et al., 1996; Niederhoffer and Szabo, 1999); although more recently, cardiac mechanisms have been invoked to explain the lowering of blood pressure (for review, see Pacher et al., 2005). By contrast, the cardiovascular effects of cannabinoids in conscious animals are more complex, and they do not support the notion that cannabinoids are depressor agents (Stein et al., 1996; Gardiner et al., 2001, 2002a,b). Indeed, in conscious animals, cannabinoids cause a pressor effect that is CB1-receptor-mediated and that is susceptible to ganglion blockade, probably due to sympathetic excitation as opposed to sympathoinhibition (Gardiner et al., 2001, 2002b). However, no recent studies have examined the cardiovascular response to THC in conscious animals; thus, it is unknown whether the hypotensive response observed to THC in anesthetized animals (Lake et al., 1997a,b) is also evident in the conscious state, and if so, whether it is associated with vasoconstriction. Therefore, the second aim of the present study was to investigate the cardiovascular response to 1 mg kg⁻¹ i.v. THC in conscious animals. The dose of THC used was chosen to have minimal behavioral effects (Bloom et al., 1997; Varvel et al., 2005) and thus to minimize any activity-related changes in cardiovascular status.

Lastly, based on our in vitro evidence that vasorelaxant responses to THC are enhanced in vessels obtained from animals treated chronically with l-NAME (see Results), the final aim of our study was to measure the cardiovascular responses to THC in conscious l-NAME-treated rats in vivo, to test the hypothesis that THC will cause vasodilatation and hypotension in l-NAME-treated hypertensive rats.

Materials and Methods

L-NAME Treatment Protocol. All procedures were approved by the University of Nottingham Ethical Review Committee, and they were performed under UK Home Office Project and Personal License Authority. Male Sprague-Dawley rats (200–250 g) were obtained from Charles River (Margate, Kent, UK) and housed in a temperature-controlled environment (20–22°C) with a 12-h light/dark cycle. They were allowed food and water ad libitum. Animals for in vitro studies were housed in pairs, whereas those used in the in vivo studies were housed individually after surgery. Animals were assigned to either water drinking (control) or l-NAME drinking (0.1 mg ml⁻¹ solution in water) groups, with water changed every 2 to 3 days. Animals were treated with l-NAME in their drinking water for 4 weeks (Dunn and Gardiner, 1995). Fluid intake was approximately 75 ml kg⁻¹ day⁻¹ giving an l-NAME dose of ~7.5 mg kg⁻¹ day⁻¹. To ensure constancy of l-NAME administration postsurgery for catheterization and throughout the 4-day experimental protocol, l-NAME was infused intravenously (7.5 mg kg⁻¹ day⁻¹), and all rats were given water to drink.

Vessel Preparation. Animals for in vitro experimentation were stunned by a blow to the back of the head, and then they were killed by cervical dislocation. The aorta, superior mesenteric artery, and mesenteric arterial bed were removed rapidly and placed into cold Krebs-Henseleit buffer (composition: 118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃, 2 mM CaCl₂, and 10 mM d-glucose). From the mesenteric arterial bed, 2-mm segments of third-order branches of the superior mesenteric artery (G3) were dissected free of adherent connective and adipose tissue. G3 vessels were mounted on fine tungsten wires (40 μm in diameter) on a Mulvany-Halpern myograph (Myo-Interface model 410A; Danish Myo Technology, Aarhus, Denmark). The superior mesenteric artery (G0; 3–4 mm in length) and thoracic aorta (4–6-mm-long segments) were also cleaned of adherent tissue and mounted on fixed segment support pins using the Multi Myograph system (model 610M; Danish Myo Technology). Tension was measured and was recorded on a MacLab 4e recording system (ADInstruments, Hastings, UK).

Once mounted, all vessels were kept at 37°C in Krebs-Henseleit buffer and gassed with 5% CO₂ in O₂. The mesenteric vessels were stretched to an optimal passive tension of 5 mN and the thoracic aorta to 10 mN tension (O’Sullivan et al., 2005). All vessels were allowed to equilibrate, and the contractile integrity of each vessel was tested by its ability to contract to 60 mM KCl by at least 5 mN. The vasorelaxant effects of THC were assessed in preparations obtained from l-NAME-treated animals compared with control animals. Arteries (G3, G0, and the aorta) were contracted with methoxamine and the thromboxane mimetic U46619 (O’Sullivan et al., 2005). Once a stable contraction was achieved, the effects of THC (100 nM–100 μM) were assessed as cumulative concentration-response curves by addition of THC to the 5-ml organ bath.

The steady-state response to THC was taken at each concentration and expressed as the percentage of relaxation of the pharmacologically induced contraction. In G3 vessels, the effects of chronic l-NAME treatment on the vasorelaxant response to other vasorelaxants, acetylcholine (endothelium-dependent), and verapamil (endothelium-independent) were also examined. The involvement of the cannabinoid CB₁ receptor was assessed using the CB₁ receptor antagonist AM251 at 1 μM (Gatley et al., 1996) added to the preparations 10 min before preconstriction (approximately 30 min before the onset of the THC dose-response curve). The involvement of TRPV1 receptors was assessed by incubating vessels for 1 h with the TRPV1 receptor agonist capsaicin at 10 μM to deplete the sensory nerves of vasoactive neurotransmitters, followed by a 20-min washout. The role of prostanoids was investigated by performing experiments in the presence of the cyclooxygenase (COX) inhibitor indomethacin at 10 μM, present throughout the experiment. It has been previously shown that l-NAME-treated animals show increased contractile responses in the mesenteric bed (Navarro-Olid et al., 1996; Ruiz-Marcos et al., 2001) and that incubation with THC reduces methoxamine-induced contractile responses (O’Sullivan et al., 2006). Therefore, we investigated the effects of THC on contrac-
tile function in arteries (G3 and G0) obtained from L-NAME-treated rats by constructing concentration-response curves to methoxamine in adjacent segments of arteries 2 h after adding either 10 μM THC or vehicle (5 μl of ethanol) to the organ baths.

**Surgical Procedures.** Two weeks after the onset of L-NAME/water drinking, animals underwent the first surgical procedure for implantation of miniaturized Doppler flow probes. Under general anesthesia (fentanyl and medetomidine at 300 μg kg⁻¹ each i.p., supplemented as required), probes were sutured around the left renal and superior mesenteric arteries and around the distal abdominal aorta below the level of the ileocecal artery, allowing measurement of hindquarters flow. Reversal of anesthesia and provision of analgesia was achieved using atipamezole and nalbuphine at 1 mg kg⁻¹ each s.c. At least 10 days after probe implantation, and subject to animals passing veterinarian checks, the second stage of surgery, catheterization, was conducted. Using the same anesthetic regimen, catheters were implanted into the distal abdominal aorta via the caudal artery for measurement of arterial blood pressure and heart rate, and three separate intravenous catheters were placed in the right jugular vein for separate drug administration. At this stage, the wires from the flow probes were soldered into a miniature plug that was fixed to a custom-designed harness worn by the rat. The catheters ran through a protective spring attached to the harness and a counterbalanced lever system, which allowed free movement for the animals in their home cage. Following surgery, the animals were left to recover for 24 h, and they were fully conscious and freely moving before experimentation, with free access to food and water. Arterial and venous catheters were connected to fluid-filled, double-channel swivels to allow overnight intra-arterial infusion of heparinized (15 U ml⁻¹; 0.4 ml h⁻¹) saline to maintain catheter patency, and intravenous infusion of saline (control rate; 0.4 ml h⁻¹) or L-NAME (7.5 mg kg⁻¹ day⁻¹). The latter approach was taken to ensure constancy of L-NAME administration in the first 24 h postsurgery, when fluid intake would be expected to be lower than normal. Thereafter, L-NAME or saline was infused intravenously for the rest of the 4-day experimental period, and all animals were given tap water to drink.

**In Vivo Cardiovascular Recordings.** Arterial catheters were connected to a fluid-filled pressure transducer, and the pressure was converted into an electrical signal that was sent via a transducer amplifier (model 13-4615-50; Gould Instrument Systems Inc., Cleveland, OH) to a customized data capture system (hemodynamics data acquisition system, HDAS; University of Limburg, Maastricht, The Netherlands). Signals from the Doppler flow probes were also recorded using HDAS via a Doppler flowmeter (VF-1 mainframe fitted with high-velocity (model HVPD-20) modules; Crystal Biotech Northborough, MA]. HDAS sampled data every 2 ms, which were averaged every cardiac cycle and stored to disc at 5-s intervals.

Experiments were performed over 4 days. On the first and second day, animals were given either 1 mg kg⁻¹ THC in 0.5 ml of vehicle over 20 min or vehicle (saline with 5% propylene glycol and 2% Tween 80) in 0.5 ml over 20 min. On both days, this was preceded by a 30-min infusion of the vehicle for AM251 (saline with 5% propylene glycol and 2% Tween 80). On the 3rd and 4th days, animals were given either THC or vehicle as before, preceded by a 30-min infusion of AM251 (3 mg kg⁻¹; 1.0 ml). Animals did not receive THC on consecutive days.

**Statistical Analysis.** The concentration of vasorelaxant giving the half-maximal response (EC₅₀) was obtained from the concentration-response curve fitted to a sigmoidal logistic equation with the minimal vasorelaxation set to zero by using Prism package (GraphPad Software Inc., San Diego, CA). Maximal responses and pEC₅₀ (negative logarithm of the EC₅₀ values) are expressed as mean ± S.E.M. The number of animals in each group is represented by n. In vitro data were compared, as appropriate, by Student’s unpaired t test or by analysis of variance (ANOVA) with statistical significance between manipulations and controls determined by Dunnett’s post hoc test.

All in vivo data were analyzed offline using Datview software (University of Limburg). Average values at selected time points were extracted into a customized statistical package (Biomed, University of Nottingham, Nottingham, UK) for subsequent analysis. Nonparametric two-way analysis of variance was used for within-group comparisons (Friedman’s test), and Mann-Whitney (unpaired) and Wilcoxon’s (paired) tests were used for between-group comparisons. A P value of < 0.05 was taken as significant.

**Drugs.** For in vitro experiments, all drugs were supplied by Sigma Chemical (Poole, Dorset, UK) except where stated. AM251 was obtained from Toecis Cookson Inc. (Bristol, UK). Acetycholine and methoxamine were dissolved in distilled water. THC, capsicain, and verapamil were dissolved in ethanol at 10 mM with further dilutions made in distilled water. Indomethacin was dissolved first in 100 μl of ethanol and then dissolved into the Krebs-Henseleit solution. AM251 was dissolved in dimethyl sulfoxide to 10 mM, with further dilutions in distilled water.

For in vivo experimentation, fentanyl citrate was purchased from Martinvale (Essex, UK); medetomidine hydrochloride (Domitor) and atipamezole hydrochloride (Antisedan) were obtained from Pfizer Central Research (Sandwich, Kent, UK); DuPont (Hounslow, UK) supplied nalbuphine hydrochloride (Nubain). L-NAME was supplied by Sigma Chemical and dissolved in tap water. AM251 and THC solutions were made in saline with 5% propylene glycol (Sigma Chemical) and 2% Tween 80 (BDH, Poole, Dorset, UK).

**Results**

**In Vitro Myography.** There was no significant difference in the level of tone imposed on arteries between control and L-NAME-treated animals (G3: control, 1.26 ± 0.09 g of tension, n = 38 versus L-NAME, 1.28 ± 0.10 g of tension, n = 34; G0: control, 1.30 ± 0.14 g of tension, n = 8 versus L-NAME, 1.45 ± 0.23 g of tension, n = 9; aorta: control, 2.64 ± 0.42 g of tension, n = 9 versus L-NAME, 2.56 ± 0.33 g of tension, n = 8). Mesenteric resistance vessels (G3) from animals treated chronically with L-NAME showed enhanced vasorelaxant responses to THC compared with control arteries (P < 0.01, n = 8; Fig. 1A; Table 1). Vasorelaxation to the endothelium-dependent vasorelaxant, acetycholine was not affected by the L-NAME-treatment (Fig. 1B). Likewise, vasorelaxation to the calcium channel blocker verapamil was not different between arteries from L-NAME-treated and control animals (Fig. 1C; Table 1).

To establish whether similar enhancements of the vasorelaxant effects of THC are seen in conduit vessels, the effects of chronic L-NAME treatment were examined on vasorelaxation to THC in the superior mesenteric artery (G0) and the aorta. In preparations taken from animals treated with L-NAME, the maximal relaxant effect of THC was enhanced in G0 vessels (L-NAME-treated Rₘₐₓ = 38.2 ± 4.9, n = 7 versus control Rₘₐₓ = 16.4 ± 2.8, n = 9; P < 0.01; Fig. 2A). In the aorta, in control animals, THC did not cause a vasorelaxant effect (5.1 ± 5.7% contraction at 100 μM THC). By contrast, in L-NAME-treated animals, THC caused a small degree of vasorelaxation, although this was not significantly different compared with control (4.0 ± 7.7% relaxation at 100 μM THC; Fig. 2B).

The involvement of the cannabinoid CB₁ receptor in THC-mediated vasorelaxation in G3 vessels was investigated using the cannabinoid CB₁ receptor antagonist AM251 at 1 μM. AM251 did not affect the vasorelaxant response to THC in arteries from either control animals (n = 7) or from L-NAME-treated animals (n = 6; Fig. 3, A and B). The involvement of sensory nerves in THC-mediated vasorelaxation in G3 ves-
significant difference between the L-NAME and control arteries from L-NAME-treated animals was significantly ant response to THC in the presence of indomethacin in the rat mesentery (G3). Data are given as means with error bars representing the mean ± S.E.M. * denotes a significant difference between control and L-NAME-treated arteries in the pEC50 obtained for the vasorelaxant compounds.

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<th>TABLE 1</th>
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<td>pEC50 values in G3 vessels from control and L-NAME-treated animals</td>
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<th>Water-Drinking Controls</th>
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<tr>
<td>Acetylcholine</td>
<td>7.64 ± 0.17</td>
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<td>Verapamil</td>
<td>7.53 ± 0.19</td>
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<tr>
<td>THC</td>
<td>5.58 ± 0.12</td>
<td>6.13 ± 0.13*</td>
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<tr>
<td>THC and AM251</td>
<td>5.87 ± 0.21</td>
<td>6.02 ± 0.16</td>
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<tr>
<td>THC and capsaicin</td>
<td>4.68 ± 0.30†</td>
<td>4.55 ± 0.25†</td>
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<tr>
<td>THC and indomethacin</td>
<td>5.82 ± 0.26</td>
<td>5.14 ± 0.20**†</td>
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* Significant difference between the control and L-NAME treated animals.
† Significant difference from control THCAEC50 values within each group.

with controls) (compare L-NAME, \( R_{max} = 1.62 ± 0.10 \) g of tension, and L-NAME and THC, \( R_{max} = 1.08 ± 0.15 \) g of tension; ANOVA, \( P < 0.05; n = 6; \) Fig. 4B).

In Vivo Cardiovascular Measurements. Resting cardiovascular variables in the two groups of animals on the four experimental days are presented in Table 2. On each day, L-NAME-treated animals had a significantly higher resting blood pressure, with tendencies for decreased vascular conductance, although this was significant only for mesenteric vascular conductance before THC infusion after vehicle infusion, and for hindquarters vascular conductance before vehicle infusion after vehicle infusion. The dose of THC used was chosen to have minimal behavioral effects (Bloom et al., 1997; Varvel et al., 2005), and although some acute effects of THC were observed on motor activity and behavior (increased locomotor activity), the cardiovascular responses to THC reported did not seem to be a result of any changes in behavior.

Hemodynamic Responses to THC. In controls, following vehicle administration (saline with 5% propylene glycol and 2% Tween 80; 0.5 ml over 20 min), there were no changes in heart rate or renal or hindquarters vascular conductances over 4 h, but there was a gradual small fall in mean arterial blood pressure (−5 ± 1 mm Hg at the end of 4 h; Friedman’s test, \( P < 0.05; \) data not shown). THC at 1 mg kg⁻¹ caused a long-lasting increase in blood pressure compared with baseline values (up to 2 h postinfusion) in control animals (Fig. 5). This was accompanied by significant decreases from baseline in renal and mesenteric vascular conductances and by an immediate and long-lasting (1-h) increase in hindquarter vascular conductance, with a small fall in hindquarters vascular conductance toward the end of the experimental period (Fig. 5). Although the fall in heart rate in response to THC in control rats was not significant (Friedman’s test, Fig. 5), the integrated (0–240 min) bradycardia was significantly greater than the effect of vehicle administration (compare THC, 6702 ± 1361, and vehicle, 1277 ± 680 beats; Wilcoxon’s test, \( P < 0.05)\).

In L-NAME-treated animals, following vehicle administration, there were also no changes in heart rate or renal, mesenteric, or hindquarters vascular conductances, but there was a significant fall in mean arterial blood pressure during the last hour of the experiment (−10 ± 2 mm Hg at the end of 4 h; Friedman’s test, \( P < 0.05; \) data not shown). THC at 1 mg kg⁻¹ caused a long-lasting increase in blood pressure in L-NAME-treated animals, accompanied by signif-
significant decreases from baseline in renal and mesenteric vascular conductances (Friedman’s test; Fig. 5). THC also caused an increase in hindquarter vascular conductance; however, this was short-lived (Fig. 5).

The effects of THC on blood pressure and mesenteric vascular conductance in L-NAME-treated animals were not significantly different from those seen in control animals (Mann-Whitney test), but the integrated (0–60-min) renal vasoconstriction was significantly smaller in L-NAME-treated rats (compare control, 785 ± 169, and L-NAME, 383 ± 116% min; Mann-Whitney test, $P < 0.05$). The integrated (0–60-min) hindquarters vasodilatation was also significantly smaller (compare control, 1586 ± 344, and L-NAME, 626 ± 103% min; Mann-Whitney test, $P < 0.05$).

Effects of CB1 Receptor Antagonism on the Hemodynamic Responses to THC. In control rats, administration
of AM 251 at 3 mg kg\(^{-1}\) i.v. had no significant effects on resting cardiovascular status over the 30 min before administration of vehicle or THC. In rats given AM251 followed by vehicle, there was a small fall in mean arterial blood pressure over time (−5 ± 2 mm Hg at the end of 4 h; Friedman’s test, \(P < 0.05\); data not shown). In rats given AM251, the integrated (0- to 60-min) pressor effect of THC over the first 60 min was diminished compared with THC alone (integrated area 0 to 240 min; compare THC, 84.5 ± 150, and THC and AM251, 38.9 ± 125 mm Hg min; Wilcoxon’s test, \(P < 0.05\)), and the renal and mesenteric vasoconstrictions and the hindquarters vasodilatation were abolished (Fig. 6A). In the presence of AM251, THC caused a significant and long-lasting bradycardia (up to 4 h postinfusion; Fig. 6A) that was significantly greater than that seen with THC alone (integrated area 0 to 240 min; compare THC, 521 ± 995, and THC and AM251, 96.67 ± 1568 beats; Wilcoxon’s test, \(P < 0.05\)). There was a small decrease in mean arterial blood pressure between 2 and 4 h after administration of THC in animals given AM251 (−9 ± 3 mm Hg at the end of 4 h, Friedman’s test, \(P < 0.05\)), but this did not differ from the changes seen with AM251 followed by vehicle administration (see above).

In L-NAME-treated animals, on one of the experimental days where AM251 was given, it caused a small but significant increase in resting blood pressure before THC administration (118 ± 3 to 125 ± 2 mm Hg after 30 min), accompanied by a decrease in mesenteric vascular conductance [from 55 ± 5 to 49 ± 4 (kHz mm Hg\(^{-1}\) \(10^3\)]. In the presence of AM251, the effects of THC on blood pressure and mesenteric vascular conductances were markedly inhibited, the hindquarters vasodilatation was abolished, and the hindquarters vasoconstriction was enhanced (Fig. 6B). There was no difference between control and L-NAME-treated rats in the response to THC after AM251 administration (Mann-Whitney test; Fig. 7).

### Discussion

This study characterizes the cardiovascular responses to THC both in vitro and in vivo in normotensive and chronic L-NAME-treated rats. Results indicate that, in vitro, isolated arteries of L-NAME-treated rats show enhanced vasorelaxation to THC due to an increased sensory nerve component and stimulation of prostanoid production but not through the CB\(_1\) receptor. Although THC caused vasorelaxation of mesenteric resistance vessels in vitro, in vivo, THC caused a CB\(_1\) receptor-mediated pressor effect and mesenteric vasoconstriction, with a CB\(_1\) receptor-mediated increase in hindquarter conductance in normotensive rats. Furthermore, in contrast to the findings in vitro, there was no evidence for enhanced vasodilator effects of THC in vivo following L-NAME treatment.

The first aim of the present study was to investigate the vascular responses to THC in isolated resistance arteries from rats treated chronically with L-NAME, on the basis that Mendizábal et al. (2001) reported that vasorelaxation to
anandamide was enhanced in perfused mesenteric beds of these animals. We found that in mesenteric arteries from control rats, THC caused modest vasorelaxation of a similar potency to that previously published (O'Sullivan et al., 2005). However, in rats treated chronically with L-NAME, the vasorelaxant response to THC was significantly enhanced. Although it has not been previously examined whether THC responses are altered after chronic NOS inhibition, these data are consistent with previous studies showing that THC causes a greater depressor effect in hypertensive than normotensive patients (Crawford and Merritt, 1979) and that THC causes hypotension in conscious SHRs but not in normotensive rats (Kosersky, 1978).

In SHRs, CB₁ receptor antagonists increase blood pressure (Batkai et al., 2004), and CB₁ receptor agonists lower blood pressure more than in normotensive rats (Lake et al., 1997; Batkai et al., 2004). Immunohistochemical evidence also shows CB₁ receptor up-regulation in the aorta of SHRs (Batkai et al., 2004). Although under normal conditions the CB₁ receptor does not participate in the vasorelaxant effects of THC in mesenteric resistance arteries (O'Sullivan et al., 2005), we examined the possibility that the enhanced vasorelaxant response to THC after L-NAME treatment was due to up-regulation of CB₁ receptor function. However, CB₁ receptor antagonism did not affect vasorelaxation to THC in either group, ruling out this possibility.

Administration of the TRPV₁ receptor agonist capsaicin leads to a greater depressor effect in SHRs than in normotensive rats (Li et al., 2003). Because it is known that THC causes vasorelaxation through the release of calcitonin gene-related peptide from sensory nerves (Zygmont et al., 2002), we investigated the role of sensory nerves in vasorelaxation to THC in L-NAME-treated animals. Capsaicin pretreatment reduced the potency of THC in both groups of animals; however, the inhibition of the THC-mediated vasorelaxant response was greater in the L-NAME-treated animals, indicating that vasorelaxation to THC through sensory nerves may be up-regulated in animals treated chronically with L-NAME, potentially through increased release of calcitonin gene-related peptide or through increased expression of the calcitonin receptor-like receptor (Li et al., 2003).

Vasorelaxation to THC in cerebral arteries is mediated via prostanoids (Ellis et al., 1995), and we investigated whether the enhanced vasorelaxation to THC in arteries from L-NAME-treated rats was due to increased prostanoid release. Although indomethacin did not affect responses in control preparations, in arteries from L-NAME-treated animals, the potency of THC was significantly reduced. COX-2 expression (and vasorelaxant prostanoid production) is enhanced in animals treated chronically with L-NAME in compensation for reduced NO (Henrion et al., 1997). Thus, prostanoid release, as a consequence of increased COX-2 expression, may be partly responsible for the increased vasorelaxant effect of THC.

Perfused whole mesenteries from L-NAME-treated animals show increased contractile reactivity (Navarro-Cid et al., 1996; Ruiz-Marcos et al., 2001), possibly due to increased calcium sensitivity (Ruiz-Marcos et al., 2001). We have previously demonstrated that preincubation with THC inhibits methoxamine responses, partly through blockade of calcium channels (O'Sullivan et al., 2006); therefore, we investigated the effect of THC on contractile responses in L-NAME-treated rats. It is noteworthy that the enhanced contractility of the superior mesenteric artery L-NAME-treated rats was reduced after 2-h incubation with THC to a level not significantly different from control, as has been shown previously with antihypertensive treatment (ACE inhibitors or calcium antagonists) (Navarro-Cid et al., 1996).

Against the background that THC causes vasorelaxation in vitro, the second aim of the present study was to establish whether THC is a depressor agent in vivo. We have shown for the first time that in conscious rats, THC causes a rise in systemic blood pressure and vasoconstriction of the renal and mesenteric vascular beds, with vasodilatation in the hindquarters mediated by CB₁. These data contrast with reports on the cardiovascular response to THC in anesthetized animals, where a sustained, CB₁ receptor-mediated, hypotensive response has been reported previously (Lake et al., 1997a). However, our data are in line with previous reports of pressor effects in response to cannabinoids in conscious rats in vivo (Gardiner et al., 2001, 2002a,b). Our data are also consistent with those of Jandhyala and Hamed (1978), who showed that the hypotensive effect of THC was observed in anesthetized but not conscious dogs. Likewise, Lake et al.
(1997b) reported a depressor effect of anandamide in anesthetized, but not conscious, rats.

The hemodynamic response profile to THC is remarkably similar to other CB1-receptor agonists, which cause renal and mesenteric vasoconstriction with hindquarters dilator actions, also susceptible to AM251, in conscious, chronically instrumented rats (Gardiner et al., 2001, 2002). We have also shown that the pressor response to WIN55212-2 in conscious animals is susceptible to neurohumoral blockade and likely to be due to sympathoexcitation (Gardiner et al., 2001). By contrast, under anesthesia, cannabinoids inhibit noradrenaline release from postganglionic sympathetic axons, causing hypotension (Varga et al., 1996; Niederhoffer and Szabo, 1999). This debate over cannabinoid-mediated sympathoexcitation versus sympathoinhibition in the conscious versus anesthetized state may be due to differences in the basal sympathetic tone, because it has been previously shown that under anesthesia, the pressor responses evoked by α-adrenergic agonists are decreased (Armstrong et al., 1982). Another possibility is that the central effects of cannabinoids might be more susceptible to inhibition by general anesthesia.

Because THC causes vasorelaxation in isolated arteries, our studies clearly show a discrepancy between the in vitro and in vivo responses to THC. The results obtained from experiments performed in vitro are largely postjunctional and are therefore not influenced by in vivo control systems such as the autonomic nervous system. As such, it seems that the CB1-receptor mediated prejunctional effects of THC (pressor effects) predominate over any direct vascular actions in vivo, although it should be noted that after CB1-receptor blockade, a hypotensive and/or vasodilator effect of THC was not revealed. However, it should also be noted that in vitro, THC causes only a modest vasorelaxant effect at relatively high concentrations, and it is possible that much larger doses of THC would be required in vivo to show a vasodilator effect in the mesenteric vascular bed. Clearly, such experiments are not feasible in conscious animals due to the profound behavioral effects of higher doses of THC.

THC has been shown to cause vagally mediated bradycardia in anesthetized animals (Kawasaki et al., 1980), and repeated THC dosing in humans leads to increased vagal activity (Benowitz and Jones, 1981). We observed that after preadministration of AM251, the bradycardic response to THC was enhanced, suggesting that CB1 receptors may be coupled to positive chronotropic activity, either centrally or directly. This would be consistent with the notion that the THC-mediated sympathoexcitation described above also involves tachycardia.

Because vasorelaxation to THC was enhanced in arteries from animals treated chronically with l-NAME, the final aim of this study was to establish whether in vivo hemodynamic responses to THC are altered in chronic l-NAME-treated rats. The most notable results from these experiments were that 1) the pressor and mesenteric vasoconstrictor responses to THC were similar to those of the control animals, 2) chronic l-NAME-treated rats showed a long-lasting bradycardic response to THC, and 3) the hindquarter vasodilator effect of THC in normotensive rats was absent in l-NAME-treated rats.
Cardiovascular Effects of THC in L-NAME-Treated Rats

It has been suggested that CB1 receptors modulate cardiovascular function in various rat models of hypertension (Batkai et al., 2004). Therefore, we investigated whether there was any role for CB1 receptors in the cardiovascular responses to THC in L-NAME-treated rats. AM251 significantly reduced the pressor effects of THC and reduced the mesenteric vasoconstriction; however, there was no evidence of a CB1 receptor-mediated depressor effect in the L-NAME-treated rats. On one of the days after AM251 was administered, the pressor responses to THC in L-NAME-treated rats, arterial pressure was slightly increased (by 7 mm Hg); however, this increase is considerably less than that reported by Batkai et al. (2004) in anesthetized hypertensive rats after CB1 receptor antagonism (~30 mm Hg increase in blood pressure).

The bradycardic response to THC was more prominent in L-NAME rats. Studies by Vasquez et al. (1994) indicated that chronic inhibition of NO in rats enhances the bradycardic component of the baroreflex. Later studies showed that this was related to increased responsiveness to acetylcholine in L-NAME-treated rats in compensation for decreased vagal activity (Araujo et al., 1998). In the context of the present study, this suggests that vagal stimulation by THC leads to a greater reduction in heart rate in chronic L-NAME-treated rats as a consequence of enhanced cardiac muscarinic receptor responsiveness.

In the present study, there was a large and long-lasting hindquarters vasodilator response to THC in control animals, sensitive to AM251, and consistent with previous data from our group for other CB1 receptor agonists (Gardiner et al., 2002). In that study, the β2-adrenoceptor antagonist ICI118551 also inhibited the effects of WIN55212-2 and HU-210, suggesting the vasodilator response to THC also involves β2-adrenoceptors. Since we observed that the hindquarter vasodilatation is greatly reduced after L-NAME-treatment, this response to THC may be mediated by NO, consistent with an involvement of NO in β2-adrenoceptor-mediated vasodilatation (for review, see Ritter et al., 2006; Gardiner et al., 1991).

In conclusion, we have shown that although THC causes vasorelaxation in vitro, in vivo, THC infusion causes a CB1-mediated pressor effect, which is likely to be due to sympathoexcitation. Likewise, despite enhanced vasorelaxant responses to THC in arteries from L-NAME-treated rats, the pressor response to THC in vivo was similar between control and hypertensive rats. Our results provide no evidence for a hypotensive effect of acute THC administration in either normotensive or L-NAME-treated, hypertensive conscious rats.

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Fig. 7. Hemodynamic responses to an infusion of 1 mg kg⁻¹ THC infused over 20 min i.v. (arrows mark the beginning and end of infusion) in the presence of AM251 at 3 mg kg⁻¹ i.v. infused over 30 min in water-drinking (●) and L-NAME-drinking (○) conscious Sprague-Dawley rats. Data are given as means with vertical bars representing the mean ± S.E.M. * denotes a significant change in variables from baseline (Friedman’s test) within each group in response to THC in the presence of AM251. Between-group comparisons based on integrated responses are given in the text.
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