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RESEARCH PAPER

Acute hypertension reveals depressor and vasodilator effects of cannabinoids in conscious rats

W-S Vanessa Ho* and Sheila M Gardiner

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

Background and purpose: The cardiovascular effects of cannabinoids can be influenced by anaesthesia and can differ in chronic hypertension, but the extent to which they are influenced by acute hypertension in conscious animals has not been determined.
Experimental approach: We examined cardiovascular responses to intravenous administration of anandamide and the synthetic cannabinoid, (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (WIN55212-2), in conscious male Wistar rats made acutely hypertensive by infusion of angiotensin II (AII) and arginine vasopressin (AVP). Rats were chronically instrumented for measurement of arterial blood pressure and vascular conductances in the renal, mesenteric and hindquarters beds.
Key results: Anandamide dose-dependently decreased the mean arterial blood pressure of rats made hypertensive by AII-AVP infusion, but not normotensive rats. Interestingly, acute hypertension also revealed a hypotensive response to WIN55212-2, which caused hypertension in normotensive animals. The enhanced depressor effects of the cannabinoids in acute hypertension were associated with increased vasodilatation in hindquarters, renal and mesenteric vascular beds. Treatment with URB597, which inhibits anandamide degradation by fatty acid amide hydrolase, potentiated the depressor and mesenteric vasodilator responses to anandamide. Furthermore, haemodynamic responses to WIN55212-2, but not to anandamide, were attenuated by the CB_1 receptor antagonist, AM251 [N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophen yl)-4-methyl-1H-pyrazole-3-carboxamide].
Conclusions and implications: These results broadly support the literature showing that the cardiovascular effects of cannabinoids can be exaggerated in hypertension, but highlight the involvement of non-CB_1 receptor-mediated mechanisms in the actions of anandamide.
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Keywords: anandamide; WIN55212-2; hypertension; fatty acid amide hydrolase; cannabinoid receptors; haemodynamic responses; conscious rats

Abbreviations: AM251, N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; URB597, 3′-carbamoyl-biphenyl-3-yl-cyclohexylcarbamate; WIN55212-2, (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone

Introduction

Emerging evidence suggests that endogenous agonists for cannabinoid receptors (endocannabinoids) act as novel lipid signalling molecules in the cardiovascular system. In particular, the archetypal endocannabinoid, anandamide, which is a partial agonist for the cannabinoid CB_1 receptor, has been shown to elicit complex cardiovascular effects *in vivo* (see Randall *et al.*, 2004; Pacher *et al.*, 2005a). Thus, anandamide can modulate vascular tone (acting as a vasodilator *in vitro*), cardiac function and neuronal output of central cardiovascular centres (Randall *et al.*, 2004; Pacher *et al.*, 2005a). Some haemodynamic responses to anandamide have been attributed to activation of CB_1 receptors (Varga *et al.*, 1996; Ledent *et al.*, 1999). However, anandamide is also an agonist for the transient receptor potential vanilloid type 1 (TRPV1) receptor on sensory nerves (Zygmunt *et al.*, 1999; Li *et al.*, 2003), and hence activation of TRPV1 receptors might also mediate some of the cardiovascular effects induced by anandamide. Of particular interest is the triphasic blood pressure response to intravenous administration of anandamide, which has been

Correspondence: Dr W-S Vanessa Ho, Division of Basic Medical Sciences (Level 1A, Jenner Wing), St George's, University of London, Cranmer Terrance, London SW17 0RE, UK. E-mail: vho@sgul.ac.uk
*Present address: Division of Basic Medical Sciences, St George's, University of London, Cranmer Terrance, London, SW17 0RE, UK.
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characterized mainly in anaesthetized animals (Varga *et al.*, 1996; Lake *et al.*, 1997a; Malinowska *et al.*, 2001a; Li *et al.*, 2003). The initial, transient bradycardia and consequent hypotension (Phase I) are thought to be mediated by vagal stimulation via activation by anandamide of TRPV1 receptors; the pressor effect (Phase II) could involve both central and peripheral mechanisms (including TRPV1 but not CB_1 receptors) (Malinowska *et al.*, 2001b; Kwolek *et al.*, 2005); the prolonged depressor response (Phase III) is considered to be largely mediated by presynaptic CB_1 receptors and subsequent inhibition of sympathetic transmission (Varga *et al.*, 1996; Jarai *et al.*, 1999; Ledent *et al.*, 1999; Malinowska *et al.*, 2001a; Pacher *et al.*, 2004).

Increased production of anandamide, acting via CB_1 receptors, has been implicated in pathophysiological conditions associated with hypotension, for instance haemorrhagic shock (Wagner *et al.*, 1997), cardiogenic shock (Wagner *et al.*, 2001) and advanced liver cirrhosis (Batkai *et al.*, 2001). Furthermore, the (Phase III) hypotensive properties of anandamide and some other CB_1 receptor agonists have led to the proposal that the cannabinoid system could offer therapeutic targets for hypertension, particularly since Kunos and co-workers have demonstrated that cannabinoid-induced falls in blood pressure are enhanced in chronically hypertensive rats, including spontaneous hypertensive rats (SHR) (Lake *et al.*, 1997a; Batkai *et al.*, 2004). However, it is noteworthy that Phase III depressor responses to anandamide or CB_1 receptor agonists are diminished or absent in normotensive conscious, as compared with anaesthetized, animals (Lake *et al.*, 1997a; Gardiner *et al.*, 2002a,b), indicating that haemodynamic profiles of cannabinoids are sensitive to experimental conditions such as anaesthesia. Furthermore, we have recently found that anandamide induced only a small reduction in blood pressure whereas the potent cannabinoid receptor agonist, (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (WIN55212-2) (Griffin *et al.*, 1998) caused a rise in blood pressure in conscious rats with various forms of chronic hypertension (rats made hypertensive by chronic inhibition of nitric oxide synthase Wheal *et al.*, 2007b), SHR (Wheal *et al.*, 2007a) and transgenic [(mRen-2)27] rats (Gardiner *et al.*, 2001). More experimentation is therefore needed to explore the interaction between the cannabinoid system and blood pressure control. In particular, it is unknown if pathological changes occurring in chronic hypertension underlie the different cardiovascular responses to cannabinoids in normotensive versus hypertensive animals.

In this study, we examined the cardiovascular effects of anandamide and WIN55212-2 in conscious, freely moving rats in acutely hypertensive conditions induced by infusion of angiotensin II (AII) and arginine vasopressin (AVP) on the day of experiment. Both AII and AVP were used as these vasoactive peptides are thought to play an important role in the development and maintenance of hypertensive states; furthermore, they provide a rapid but sustained elevation in arterial blood pressure in our model. To ascertain the contribution of changes in vascular tone to the blood pressure responses, regional blood flow (in renal, mesenteric and hindquarters vascular beds), arterial blood pressure and heart rate were monitored simultaneously. The involvement of CB_1 receptors in the responses to anandamide and WIN55212-2 were investigated by using the CB_1 receptor-selective antagonist, N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophen yl)-4-methyl-1H-pyrazole-3-carboxamide (AM251) (Lan *et al.*, 1999). The fatty acid amide hydrolase (FAAH) has been identified as the primary enzyme for the degradation of anandamide (Cravatt *et al.*, 1996), and inhibition of FAAH can enhance and/or prolong the effects of anandamide *in vivo* (Kathuria *et al.*, 2003; Batkai *et al.*, 2004; Pacher *et al.*, 2005b). Thus, modulation of FAAH activity might serve as a useful approach to target endocannabinoid signalling, especially in areas where there is a significant, basal level of endogenous anandamide. While little is known about the regulation of FAAH (Ho and Hillard, 2005), it is possible that anandamide metabolism is altered in hypertensive states and thus contributes to the differential haemodynamic responses in normotensive versus hypertensive conditions. Therefore, the effects of inhibiting FAAH on baseline cardiovascular variables and on responses to anandamide were also examined by using 3′-carbamoyl-biphenyl-3-yl-cyclohexylcarbamate (URB597), a selective FAAH inhibitor (Kathuria *et al.*, 2003) in both normotensive and acutely hypertensive rats.

Our results demonstrated that hypotensive and vasodilator responses to cannabinoids were exaggerated in conscious rats made acutely hypertensive by infusion of AII and AVP. Furthermore, while the haemodynamic effects of WIN55212-2 were mediated by CB_1 receptors, the cardiovascular effects of anandamide, at the doses used here, did not appear to involve CB_1 receptors but were modulated by inhibition of FAAH-mediated degradation.

Methods

Animals

All procedures were approved by the University of Nottingham Ethical Review Committee and were performed under UK Home Office Licence Authority. Male Wistar rats ($n = 36$; 350–450 g; Charles River, UK) were housed in a temperature-controlled environment (20–22°C) with a 12 h light/dark cycle (lights on at 06.00 h) with free access to food (Teklad global 18% protein rodent diet, Harlan UK) and water. The rats were held within the Biomedical Service Unit at the University of Nottingham for at least a week before surgery.

Surgical preparation

Surgery was carried out under general anaesthesia (fentanyl and medetomidine, 300 μg kg^{-1} of each, i.p.), which was reversed by buprenorphine (0.02 mg kg^{-1}, s.c.) and atipamezole (1 mg kg^{-1}, s.c.), with buprenorphine also providing analgesia. Procedures for cardiovascular measurements were carried out as described previously (Wheal *et al.*, 2007b). Briefly, miniaturized pulsed Doppler flow probes were sutured around the left renal and superior mesenteric arteries, and around the distal abdominal aorta (to monitor hindquarters flow). At least 10 days later, under anaesthesia (as above), catheters were implanted in the distal abdominal aorta (via the ventral caudal artery), for monitoring arterial blood

pressure and heart rate, and in the right jugular vein for drug administration. Following at least 24 h recovery from catheterization, when the animals were fully conscious and freely moving, experiments (described below) were commenced.

Blood pressure, heart rate and Doppler shift signals (an indicator of blood flow) were recorded by using a customized data capture system (Haemodynamics Data Acquisition System; University of Limburg, Maastricht, Netherlands) via a Gould transducer amplifier (Model 13-4615-50; Ohio, USA) and a Doppler flowmeter [Crystal Biotech VF-1 mainframe fitted with high velocity (HVPD-20) modules, Holliston, USA].

Experimental protocols

The experimental protocols used are illustrated in Figure 1. After a baseline period of 30–45 min, rats were continuously infused (at 0.4 mL h^{-1}, i.v.) with either saline or AII (500 ng kg^{-1} h^{-1}) plus AVP (50 ng kg^{-1} h^{-1}). In the first series of experiments, URB597 (3 mg kg^{-1}) or its vehicle was administered (i.v. infusion at 2 mL h^{-1} over 30 min) 45 min later. After a further 30 min, a bolus injection (0.12 mL; i.v.) of anandamide (1 or 3 mg kg^{-1}) was given. All experiments were run over 4 days. One group of animals was given URB597 on each of the 4 days, and the other group was given vehicle. A single animal in each group was exposed to a single dose of anandamide, under normotensive or hypertensive conditions, on each day and allowed a 24 h recovery period between experiments.

In the second series of experiments, infusion of URB597 was replaced by infusion of AM251 (3 mg kg^{-1}), which was then followed by a bolus injection of 3 mg kg^{-1} anandamide or 150 μg kg^{-1} WIN55212-2 (Fig. 1). All experiments were run over 4 days. As before, one group of animals was given AM251 on each of the 4 days and the other group was given vehicle, and a single animal was exposed to a single dose of anandamide or WIN55212-2, under normotensive or hypertensive conditions, on each day and allowed a 24 h recovery period between experiments. Doses of cannabinoids selected in this study were based on our earlier studies, which demonstrated dose-dependent haemodynamic responses to anandamide (75 μg kg^{-1}–3 mg kg^{-1}) and WIN55212-2 (5–250 μg kg^{-1}) in conscious rats (Gardiner *et al.*, 2001; 2002a; Wheal *et al.*, 2007b). These studies also indicated that vehicle for the cannabinoids has no consistent effect on the cardiovascular variables measured.

Data and statistical analysis

Data were analysed off-line by using software (Datview; University of Limburg, Maastricht, Netherlands), which interfaced with Haemodynamics Data Acquisition System. Effects of AII-AVP and saline infusion were measured by averaged values over the 15 min period before and 45 min after administration. Responses to URB597, AM251 and their vehicle were measured as the 1 min average values immediately before and, at 10 min intervals, following administration.

Responses to WIN55212-2 were measured before and at 10, 30, 60, 120, 180, 240, 300, 360, 480, 600, 900 and 1800 s post dosing. Because anandamide elicited very rapid and more transient haemodynamic effects, additional data points were obtained within 60 s of administration; responses were measured before and at 5, 10, 20, 30, 40, 50, 60, 120, 300, 600, 900 and 1800 s post dosing. Vascular conductances were calculated from the mean arterial blood pressure and Doppler shift (flow) data. Comparisons of the effects of the cannabinoids, URB597 and AM251 were performed on the integrated areas under or over the curves. The Friedman's test (non-parametric version of two-way analysis of variance allowing for multiple comparisons; Theodorsson-Norheim, 1987) was used for within-group analysis, and Mann–Whitney *U*-tests were used for between group comparisons. $P \leq 0.05$ was considered statistically significant.

Drugs

Angiotensin II and AVP were obtained from Bachem (St Helens, UK). Anandamide [supplied in Tocrisolve 100™, a soya oil-water (1:4) emulsion] was obtained from Tocris Bioscience (Bristol, UK). URB597 (Cayman Chemical, Ann Arbor, USA), AM251 and WIN55212-2 (Tocris) were mixed and suspended in sterile saline containing 5% v/v propylene glycol (Sigma Chemical Co, Poole, UK) and 2% v/v Tween 80 (BDH, Poole, UK) (with 5 min sonication). Fentanyl citrate was purchased from Martindale Pharmaceutical (Essex, UK); medetomidine hydrochloride (Domitor) and atipamezole hydrochloride (Antisedan) were obtained from Pfizer (Kent, UK); Buprenorphine (Vetergesic) was supplied by Alstoe Animal Health (York, UK). Drug and molecular target nomenclature conforms to the British Journal of Pharmacology Guide to Receptors and Channels (Alexander *et al.*, 2008).

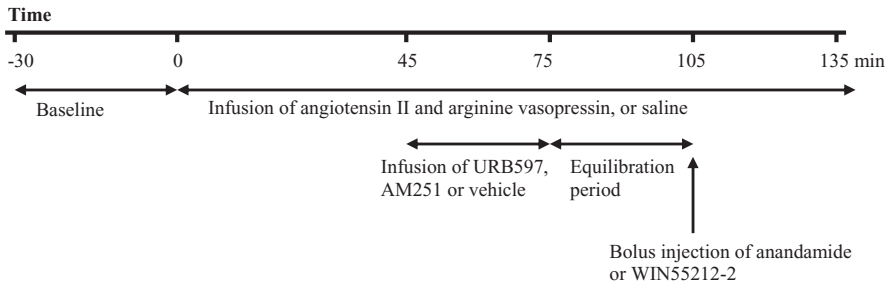

Figure 1 Experimental protocols. AM251, N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; URB597, 3'-carbamoyl-biphenyl-3-yl-cyclohexylcarbamate; WIN55212-2, (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone.

Results

Cardiovascular variables in normotensive and hypertensive, conscious rats

Similar basal cardiovascular variables were obtained in the two series of experiments, so data for treatment with AII-AVP versus saline were pooled and compared ($n = 36$). Infusion of AII-AVP significantly ($P < 0.01$, Mann–Whitney *U*-test) increased mean arterial blood pressure (149 ± 1 vs. 113 ± 1 mm Hg), reduced vascular conductance in renal [44.9 ± 1.7 vs. 76.0 ± 2.2 (kHz mm Hg^{-1})10^3], mesenteric [22.2 ± 1.1 vs. 72.4 ± 2.6 (kHz mm Hg^{-1})10^3] and hindquarters [17.8 ± 0.9 vs. 42.9 ± 1.4 (kHz mm Hg^{-1})10^3] beds and reduced heart rate (234 ± 5 vs. 368 ± 4 beats min^{-1}).

Cardiovascular responses to anandamide under normotensive and hypertensive conditions

In normotensive rats, 1 mg kg^{-1} anandamide induced a transient increase in the mean arterial blood pressure (maximum at 10 s, +18 ± 5 mm Hg) without any concomitant change in heart rate (Fig. 2A). Insignificant vasoconstrictor responses in the renal and mesenteric beds (maximum fall in conductance at 10 s, –11 ± 8 and –12 ± 4 % respectively) were also observed. In the hindquarters vascular bed, there was an initial constriction (maximum fall in conductance at 5 s, –20 ± 5%), followed by dilatation (maximum increase in conductance at 50 s, +12 ± 9%; not significant) and then constriction (maximum fall in conductance at 30 min, –14 ± 4%; Fig. 2A). However, in rats made hypertensive by AII-AVP infusion, 1 mg kg^{-1} anandamide slightly reduced the mean arterial blood pressure (at 40 s, –5 ± 2 mm Hg; Fig. 2B) and transiently increased the heart rate (maximum at 20 s, +34 ± 15 beats min^{-1}). It also caused significant increases in vascular conductance in all vascular beds without an initial decrease (renal: maximum at 30 min, +13 ± 10%; mesenteric: maximum at 30 min, +15 ± 12%; hindquarters: maximum at 30 s, +94 ± 26%; Fig. 2B). Anandamide-induced hindquarters vasodilatation was more pronounced in hypertensive compared with normotensive rats [$P < 0.01$, Mann–Whitney *U*-test on integrated responses (0–30 min); c.f. Fig. 2A,B].

In normotensive rats, the higher dose of anandamide (3 mg kg^{-1}) caused a marked bradycardia (maximum at 10 s, –157 ± 34 beats min^{-1}; Fig. 3A). In some animals, this was accompanied by a fall in blood pressure, however due to variability no significant change was obtained with the mean data. However, there was significant vasoconstriction in all vascular beds (maximum fall in conductance at 10 s, renal: –17 ± 7%; mesenteric: –46 ± 7%; hindquarters: –56 ± 7%; Fig. 3A). For both the renal and hindquarters beds, this was followed by significant increase in vascular conductance (renal: maximum at 60 s, +12 ± 4%; hindquarters: maximum at 50 s, +51 ± 11%; Fig. 3A). In rats made hypertensive by AII-AVP infusion, the bradycardic effect of anandamide was not significant (Fig. 3B), whereas mean arterial blood pressure showed a transient fall (at 5 s, –18 ± 5 mm Hg), followed by recovery to baseline (at 10 s, –0 ± 6 mm Hg) and then a more

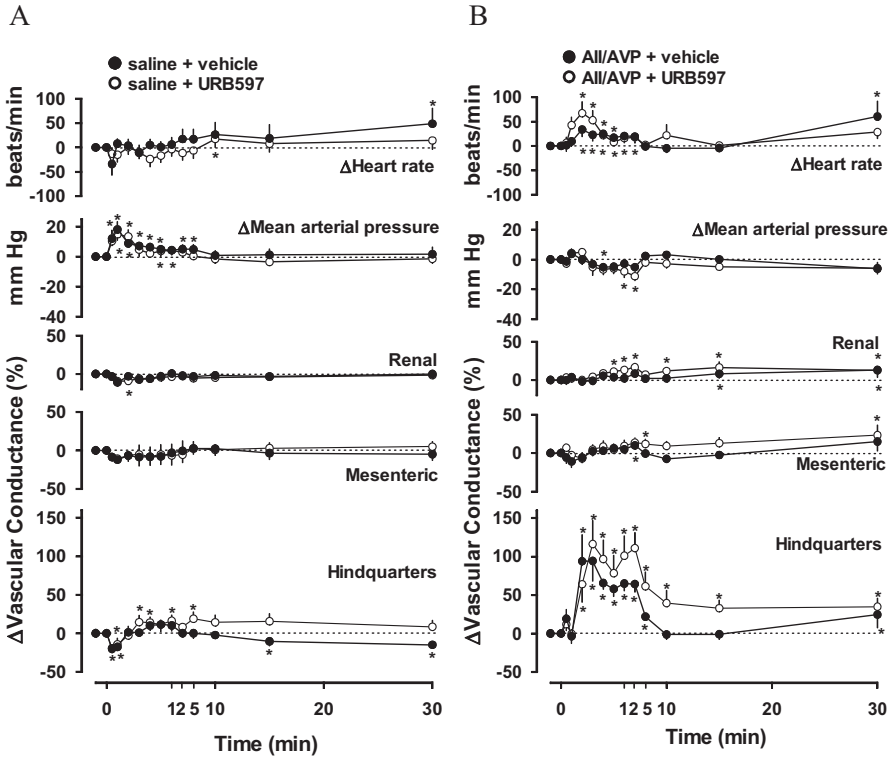

Figure 2 Haemodynamic effects of anandamide (1 mg kg^{-1}) after treatment with vehicle or URB597 (3 mg kg^{-1}) in conscious, saline-treated (A) and AII-AVP-treated (B) rats. Values are mean and vertical bars indicate SEM ($n = 7–9$). *Significant changes vs. baseline, $P \leq 0.05$ (Friedman's test). Note that a non-linear time scale is used for the initial 10 min to illustrate the rapid and transient haemodynamic effects of anandamide. AII-AVP, angiotensin II and arginine vasopressin; URB597, 3′-carbamoyl-biphenyl-3-yl-cyclohexylcarbamate.

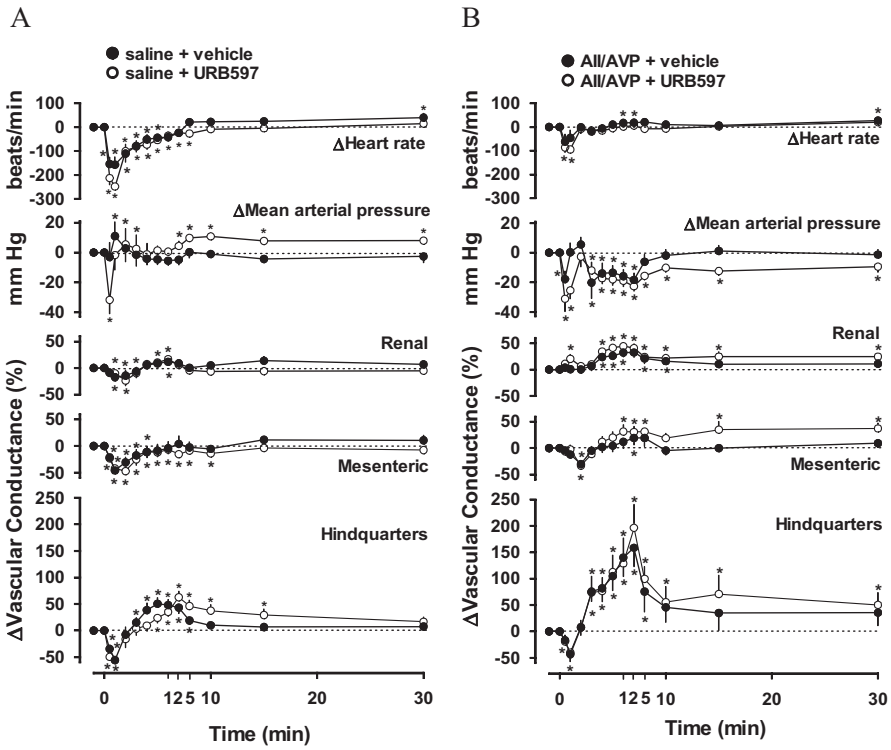

Figure 3 Haemodynamic effects of anandamide (3 mg kg^{-1}) after treatment with vehicle or URB597 (3 mg kg^{-1}) in conscious, saline-treated (A) and AII-AVP-treated (B) rats. Values are mean and vertical bars indicate SEM (n = 8–9). *Significant changes vs. baseline, $P \leq 0.05$ (Friedman's test). Note that a non-linear time scale is used for the initial 10 min to illustrate the rapid and transient haemodynamic effects of anandamide. AII-AVP, angiotensin II and arginine vasopressin; URB597, 3′-carbamoyl-biphenyl-3-yl-cyclohexylcarbamate.

sustained fall (maximum at 40 s, –20 ± 11 mm Hg; Fig. 3B). The recovery phase of the blood pressure response coincided with significant vasoconstrictions in mesenteric (maximum fall in conductance at 20 s, –30 ± 7%) and hindquarters vascular beds (maximum at 10 s, –43 ± 14%; Fig. 3B), whereas the delayed hypotension coincided with marked vasodilatation in hindquarters and, to a lesser extent, renal and mesenteric beds (all reached maximum increase in conductance at 2 min, +159 ± 36%, +32 ± 9% and +19 ± 13% respectively; Fig. 3B). As was observed at 1 mg kg^{-1}, 3 mg kg^{-1} anandamide caused a greater hindquarters dilator response under hypertensive compared with normotensive conditions [$P < 0.05$, Mann–Whitney *U*-test on integrated responses (0–30 min); c.f. Fig. 3A,B]. It was also found that the initial, renal vasoconstrictor effect of 3 mg kg^{-1} anandamide was absent in rats infused with AII-AVP [$P = 0.05$, Mann–Whitney *U*-test on integrated responses (0–30 min); Fig. 3B].

Effect of URB597 on cardiovascular responses to anandamide

The selective FAAH inhibitor, URB597 alone had no consistent cardiovascular effect compared with its vehicle in either normotensive or hypertensive conditions (Table 1). After treatment with URB597, 1 mg kg^{-1} anandamide induced similar haemodynamic responses to those seen in the absence of URB597 but with significantly reduced hindquarters vasoconstriction [$P < 0.05$, Mann–Whitney *U*-test on integrated responses (0–30 min)] in both normotensive (Fig. 2A) and hypertensive rats (Fig. 2B). Under hypertensive conditions, URB597 also slightly enhanced the hypotensive and vasodilator responses in renal and mesenteric beds [$P \leq 0.05$, Mann–Whitney *U*-test on integrated responses (0–15 min); Fig. 2B].

In normotensive rats, URB597 did not significantly affect the bradycardia induced by 3 mg kg^{-1} anandamide (Fig. 3A), but there was a transient fall in mean arterial blood pressure, followed by a sustained elevation of blood pressure by approximately 11 mm Hg, and the resultant integrated response (0–30 min) was significantly different from that obtained after vehicle treatment ($P < 0.05$, Mann–Whitney *U*-test; Fig. 3A). URB597 also significantly prolonged anandamide-induced mesenteric vasoconstriction, as well as hindquarters vasodilatation [$P < 0.05$, Mann–Whitney *U*-test on integrated responses (0–30 min); Fig. 3A].

In contrast, in rats made hypertensive by AII-AVP infusion, URB597 significantly prolonged the delayed hypotension (lasting for at least 30 min) without significantly affecting the bradycardia induced by 3 mg kg^{-1} anandamide [$P < 0.05$ Mann–Whitney *U*-test on integrated responses (0–30 min); Fig. 3B]. This was accompanied by increased vasodilatation in the mesenteric, but not renal and hindquarters, vascular beds [$P < 0.05$, Mann–Whitney *U*-test on integrated responses (0–30 min); Fig. 3B].

It was noted that within 1 min of administration, 3 mg kg^{-1} anandamide often elicited behavioural effects (a brief increase in activity including circling behaviour; not quantified) in rats treated with URB597 but not its vehicle. This was observed in both normotensive and acutely hypertensive rats.

Table 1 Basal cardiovascular variables before and 60 min after administration of URB597 ($n = 18$) or AM251 ($n = 15$–16)

	Saline		*AII-AVP*		*Saline*		*AII-AVP*	
	Baseline	*URB597*	*Baseline*	*URB597*	*Baseline*	*AM251*	*Baseline*	*AM251*
Heart rate (beats min^{-1})	361 ± 7	356 ± 8*	215 ± 7†	210 ± 6†	373 ± 12	366 ± 9	243 ± 11†	232 ± 12†
Mean arterial blood pressure (mm Hg)	114 ± 3	113 ± 3*	150 ± 2†	151 ± 2†	116 ± 2	117 ± 3	151 ± 2†	154 ± 2†
Renal VC [(kHz mm Hg^{-1})10^3]	81.7 ± 5.8	85.7 ± 7.1	49.3 ± 4.7†	50.5 ± 4.8†	71.8 ± 5.1	76.5 ± 6.1	43.8 ± 2.6†	43.0 ± 2.6†
Mesenteric VC [(kHz mm Hg^{-1})10^3]	79.4 ± 5.3	79.0 ± 5.1	21.4 ± 2.0†	21.8 ± 1.6†	68.5 ± 6.4	70.2 ± 6.7	23.1 ± 2.4†	21.4 ± 1.8†
Hindquarters VC [(kHz mm Hg^{-1})10^3]	40.4 ± 3.7	38.1 ± 3.0	17.1 ± 1.8†	14.0 ± 1.5†	42.6 ± 3.2	44.7 ± 3.8	16.4 ± 1.6†	14.3 ± 1.3†

Data are shown as mean ± SEM.
AII-AVP, angiotensin II and arginine vasopressin; AM251, N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophen yl)-4-methyl-1H-pyrazole-3-carboxamide; URB597, 3′-carbamoyl-biphenyl-3-yl-cyclohexylcarbamate; VC, vascular conductance.
*$P < 0.05$ vs. baseline within group (Friedman's test).
†$P < 0.01$ vs. corresponding value in saline group (Mann–Whitney *U*-test).

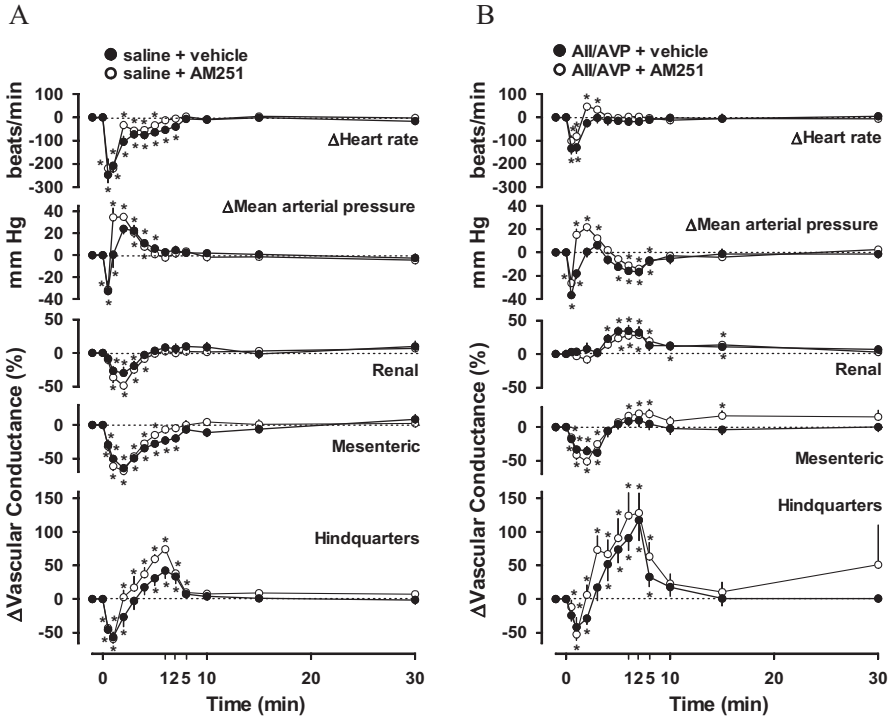

Figure 4 Haemodynamic effects of anandamide (3 mg kg^{-1}) after treatment with vehicle or AM251 (3 mg kg^{-1}) in conscious, saline-treated (A) and AII-AVP-treated (B) rats. Values are mean and vertical bars indicate SEM ($n = 7$–9). *Significant changes vs. baseline, $P \leq 0.05$ (Friedman's test). Note that a non-linear time scale is used for the initial 10 min to illustrate the rapid and transient haemodynamic effects of anandamide. AII-AVP, angiotensin II and arginine vasopressin; AM251, N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophen yl)-4-methyl-1H-pyrazole-3-carboxamide.

Effect of AM251 on cardiovascular responses to anandamide

The haemodynamic responses to 3 mg kg^{-1} anandamide under normotensive and hypertensive conditions were replicated in the second series of experiments (Fig. 4A,B), although in these experiments, blood pressure data in normotensive rats were less variable and a significant transient, fall in blood pressure, followed by a pressor effect was observed (Fig. 4A).

The CB_1 receptor-selective antagonist, AM251 alone had no consistent cardiovascular effects compared with its vehicle under either normotensive or hypertensive conditions, such that similar basal cardiovascular variables were obtained before and 60 min after administration of AM251 (Table 1). In normotensive rats, similar anandamide responses were obtained after treatment with AM251 or its vehicle, although there was a tendency for a more rapid pressor effect (Fig. 4A). In hypertensive rats, AM251 revealed a significant pressor response to anandamide without affecting the transient, or the delayed, hypotension [$P < 0.05$, Mann–Whitney *U*-test on integrated responses (0–5 min); Fig. 4B]. No significant effects

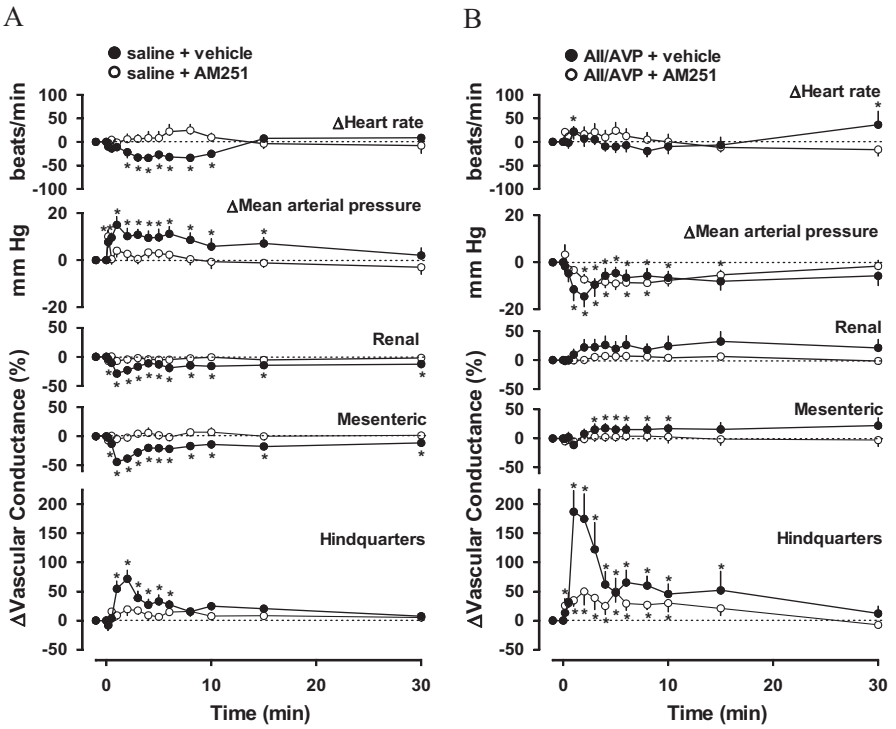

Figure 5 Haemodynamic effects of WIN55212-2 (150 μg kg^{-1}) after treatment with vehicle or AM251 (3 mg kg^{-1}) in conscious, saline-treated (A) and AII-AVP-treated (B) rats. Values are mean and vertical bars indicate SEM (n = 8–9). *Significant changes vs. baseline, $P \leq 0.05$ (Friedman's test). AII-AVP, angiotensin II and arginine vasopressin; AM251, N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophen yl)-4-methyl-1H-pyrazole-3-carboxamide.

of AM251 on anandamide-induced changes in heart rate or vascular conductances were measured (Fig. 4B).

Cardiovascular responses to WIN55212-2 under normotensive and hypertensive conditions

In normotensive rats, the synthetic cannabinoid, WIN55212-2, caused a significant bradycardia (maximum at 4 min, –34 ± 7 beats min^{-1}; Fig. 5A), which subsided within 15 min. It also increased mean arterial blood pressure by approximately 11 mm Hg, which coincided with vasoconstrictions in renal and mesenteric beds (maximum fall in conductance at 1 min for both, –29 ± 5% and –44 ± 5% respectively) and hindquarters vasodilatation (maximum increase in conductance at 2 min, +72 ± 15%; Fig. 5A). In contrast, in rats made hypertensive by AII-AVP infusion, WIN55212-2 caused slight increases in heart rate (at 1 min, +21 ± 16 beats min^{-1}) and sustained hypotension (maximum at 2 min, –15 ± 5 mm Hg; lasting for at least 15 min; Fig. 5B). The depressor response was accompanied by increases in vascular conductance in the mesenteric and hindquarters vascular beds (mesenteric: maximum at 4 min, +17 ± 8%; hindquarters: maximum at 1 min, +187 ± 37%). Although there was no significant renal vasodilator response to WIN55212-2 under these conditions (Friedman's test), the integrated changes in all cardiovascular variables were significantly different from those obtained in normotensive rats [$P < 0.05$, Mann–Whitney U-test on integrated responses (0–10 min); c.f. Fig. 5A,B].

It was noted that WIN55212-2 often induced a brief increase in activity, including circling behaviour, followed by decreased locomotion (not quantified) within minutes of administration. This was observed in both normotensive and acutely hypertensive rats.

Effect of AM251 on cardiovascular responses to WIN55212-2

AM251 significantly inhibited the haemodynamic responses, that is, bradycardia, pressor effect, vasoconstriction and vasodilatation, to WIN55212-2 in normotensive rats [$P < 0.05$, Mann–Whitney U-test on integrated responses (0–10 min); Fig. 5A]. In hypertensive rats, AM251 significantly attenuated the hindquarters vasodilator response to WIN55212-2 [$P < 0.01$, Mann–Whitney U-test on integrated responses (0–10 min); Fig. 5B] and also tended to inhibit vasodilatations in renal and mesenteric beds (Fig. 5B). Interestingly, although AM251 tended to slow the development of WIN55212-2-induced hypotension, the overall blood pressure response was not significantly different between vehicle- and AM251-treated rats (Fig. 5B).

Treatment with AM251 also appeared to reduce the occurrence of the brief, behavioural changes induced by WIN55212-2 (not quantified), in both normotensive and acute hypertensive rats.

Discussion

This study demonstrates that in conscious rats made acutely hypertensive by infusion of AII and AVP, cannabinoids can

induce a fall in blood pressure, which is accompanied by enhanced vasodilatation. Under these conditions, while some of the effects of WIN55212-2 are mediated by CB_1 receptors, the haemodynamic effects of anandamide are not CB_1 receptor-mediated but are modulated by inhibition of FAAH-mediated degradation.

In normotensive Wistar rats, anandamide predominantly increased mean arterial blood pressure which, at the higher dose of anandamide, was preceded by bradycardia and transient hypotension. These results are consistent with our previous studies in normotensive, Sprague-Dawley rats and Wistar-Kyoto rats (Gardiner *et al.*, 2002a; Wheal *et al.*, 2007b), but contrast with the triphasic blood pressure response to anandamide reported by others in normotensive, anaesthetized rats or mice (Varga *et al.*, 1995; Lake *et al.*, 1997b; Pacher *et al.*, 2005b), the third phase of which being a depressor effect. The prolonged depressor response in anaesthetized animals is thought to be due to inhibition of peripheral sympathetic transmission, probably by activation of the CB_1 receptor (Ishac *et al.*, 1996; Varga *et al.*, 1996; Lake *et al.*, 1997a; Malinowska *et al.*, 2001a). It has been suggested that a lower resting sympathetic tone in the conscious state (Carruba *et al.*, 1987) reduces the delayed depressor effect, but not the preceding pressor effect, of anandamide (Lake *et al.*, 1997b; Kwolek *et al.*, 2005), and that an elevated sympathetic tone, for example in some forms of hypertension (Guyenet, 2006), could enhance the depressor effect of anandamide. Indeed, studies have reported that the anandamide-induced hypotension (in Phase III) is present in conscious SHR (Lake *et al.*, 1997b; Wheal *et al.*, 2007a), or is more pronounced in anaesthetized rats with chronic hypertension (e.g. SHR; Batkai *et al.*, 2004, rats with chronic AII infusion over 10 days; Batkai *et al.*, 2004 and rats fed a high-salt diet; Wang *et al.*, 2005). However, it is unclear if the same is true for animals with acute hypertension. Here, for the first time, we have demonstrated that anandamide causes a dose-dependent delayed (Phase III) fall in blood pressure in conscious rats made acutely hypertensive with AII-AVP infusion. Thus, in AII-AVP-induced hypertension, anandamide (at 3 mg kg^{-1}) appeared to induce a triphasic blood pressure response; the initial, transient drop in blood pressure (Phase I) was followed by a brief recovery to baseline or a significant pressor effect (Phase II), and then a more sustained depressor response (by up to 20 mm Hg; Phase III). These results are in agreement with blood pressure responses to anandamide reported in earlier studies by using chronically hypertensive rats (Lake *et al.*, 1997b; Batkai *et al.*, 2004; Wang *et al.*, 2005), indicating that long-term compensatory or pathological changes in hypertension are not a prerequisite for the enhanced haemodynamic effects of anandamide. Thus, this finding may lend support to the proposal to target endocannabinoid signalling in various forms of hypertension.

Our data also showed that the Phase III blood pressure response to anandamide in acute hypertension was, at least in part, due to an enhanced peripheral vasodilator effect (in the hindquarters and, to a lesser extent, renal vascular beds). This is perhaps not surprising as anandamide is a well-known vasodilator *in vitro* (e.g. renal artery: Deutsch *et al.*, 1997, mesenteric artery: Zygmunt *et al.*, 1999 and thoracic aorta: O'Sullivan *et al.*, 2005). *In vivo*, several studies have also suggested anandamide-induced vasodilatation (Ellis *et al.*, 1995; Batkai *et al.*, 2001; Gardiner *et al.*, 2002a; Movahed *et al.*, 2005), although a more complex vasoactive profile is often seen and vasoconstrictor responses to anandamide are also documented (Gardiner *et al.*, 2002a; Wheal *et al.*, 2007a). However, it is unclear whether the observed vasodilatation and fall in blood pressure was due to sympathetic withdrawal (see above), because sympathoexcitation caused by AII infusion (Dendorfer *et al.*, 2002; Xu and Sved, 2002) is likely counteracted by a baroreflex-mediated reduction in sympathetic activity (Xu and Sved, 2002) in the acute hypertension model. AVP has also been shown to reduce sympathetic activity via enhanced baroreflex responses *in vivo* (Cowley *et al.*, 1984). Given that both AII and AVP also induce vasoconstrictions independently of the sympathetic system, it is feasible that greatly increased systemic vasoconstriction achieved by combined AII and AVP infusion exaggerates the vasodilator effect of anandamide, leading to a significant reduction in blood pressure. On the other hand, recent evidence suggests that reductions in cardiac function also play a major role in the depressor effect of anandamide (Batkai *et al.*, 2004; Pacher *et al.*, 2005b). Cardiac contractility was not directly measured in this study, and hence we cannot exclude the possibility of a reduced cardiac contractility and hence cardiac output contributing to the delayed, depressor effect of anandamide. It also remains to be determined whether the sustained reduction in basal heart rate in our acute hypertension model, probably due to baroreflex activation, was a contributing factor to the haemodynamic responses to anandamide. In considering the effects of anandamide in cardiovascular control in hypertension, it is important to note that differences in the degradation of anandamide could also play a role. Anandamide is primarily metabolized by FAAH-mediated hydrolysis and inhibition of FAAH, by pharmacological or genetic interventions, has been shown to enhance cardiovascular effects of anandamide *in vitro* (Mendizabal *et al.*, 2001; Ho and Randall, 2007) and *in vivo* (Pacher *et al.*, 2005b). Interestingly, however, expression of FAAH is reportedly increased in the cardiac tissues of SHR as compared with their normotensive controls, which would reduce anandamide responses but result in an enhanced effect of the FAAH inhibitor, URB597 in SHR (Batkai *et al.*, 2004). Here, in AII-AVP-induced acute hypertension, URB597 significantly prolonged the depressor effect and mesenteric vasodilatation induced by anandamide, suggesting that degradation by FAAH limits the cardiovascular effects of anandamide. This is consistent with a receptor-mediated mechanism of action for anandamide.

It should be pointed out that certain components of the haemodynamic responses to anandamide were not significantly modulated by URB597 under the current experimental conditions. In acute hypertension, vasodilator effects of anandamide in the mesenteric, but not the renal or hindquarters beds, were significantly influenced by FAAH inhibition, perhaps reflecting local FAAH activity in mesenteric arteries (Ho and Randall, 2007). Interestingly, URB597 treatment in normotensive rats failed to reveal a depressor response or mesenteric vasodilatation to anandamide. In fact, after URB597 treatment, 3 mg kg^{-1} anandamide induced a sustained rise in blood pressure (by up to 11 mm Hg) by

yet-to-be-identified mechanisms. One possibility is that metabolites that result from hydrolysis of anandamide contribute to an underlying hypotensive effect in normotensive rats. Taken together, these results indicate that potential differences in FAAH activity are unlikely to explain the increased depressor and vasodilator effects of anandamide in AII-AVP-induced hypertension. Furthermore, the lack of consistent haemodynamic effects following application of URB597 (3 mg kg^{-1}, i.v. infusion) also argues against the presence of a significant influence of endogenous anandamide on cardiovascular function. In this study, limited solubility prevented use of a higher dose of URB597; nevertheless, our data contrast with those reported by Batkai *et al.* (2004), who showed that URB597 alone ($ED_{50\%}$ = 1.7 mg kg^{-1}; maximally effective at 10 mg kg^{-1}, i.v. bolus injection) could elicit prolonged reductions in blood pressure in hypertensive, but not normotensive, anaesthetized rats. Therefore, at least in conscious animals, a higher plasma level of URB597 may be required to obtain measurable responses to endogenously produced anandamide, as compared with anandamide applied exogenously.

To further investigate mechanisms underlying the differential cardiovascular effects of anandamide in normotensive and hypertensive rats, we also examined the haemodynamic effects of the synthetic cannabinoid, WIN55212-2, and the CB_1 receptor antagonist, AM251. As reported previously (Gardiner *et al.*, 2002b; Wheal *et al.*, 2007a), WIN55212-2 caused an increase in blood pressure of normotensive rats, which was accompanied by bradycardia, vasoconstriction in renal and mesenteric beds, and hindquarters vasodilatation. In striking contrast, in AII-AVP-induced hypertension, WIN55212-2 induced prolonged hypotension, with vasodilatation in renal and mesenteric beds, as well as enhanced hindquarters vasodilatation. Furthermore, except for the depressor effect under hypertensive conditions, haemodynamic responses to WIN55212-2 in both groups of rats were almost abolished by AM251, suggesting the involvement of CB_1 receptors. Treatment with AM251 also tended to attenuate the maximal reduction in blood pressure (within 5 min of administration), but not the overall depressor effect, induced by WIN55212-2 under hypertensive conditions, possibly due to the incomplete blockade by AM251 of hindquarters vasodilatation (c.f. Fig. 5B; Gardiner *et al.*, 2002b). Nevertheless, the possibility that activation of non-CB_1 receptors also contribute to WIN55212-2 responses, as has been suggested in the brain, cannot be excluded (Breivogel *et al.*, 2001). Based on studies in conscious rabbits (Niederhoffer and Szabo, 1999; 2000), WIN55212-2 might induce opposing cardiovascular changes via CB_1 receptors: peripheral, presynaptic inhibition of noradrenaline release versus central sympathoexcitation, and it is conceivable that the balance shifted towards an overall hypotensive and vasodilator response in AII-AVP-induced hypertension. As indicated above for anandamide, the effect of acute AII-AVP infusion on sympathetic activity is unknown, hence the extent to which the effects of cannabinoids are direct or due to a modulation in sympathetic activity remains to be determined.

Analysis of homeostatic mechanisms and their interaction with cannabinoids during AII-AVP-induced hypertension is beyond the scope of this study. Nonetheless, an important finding here is that WIN55212-2 and anandamide elicited a similar pattern of haemodynamic effects under hypertensive conditions, but appeared to act via distinct mechanisms. In contrast to the case for WIN55212-2, AM251 had minimal influence on the haemodynamic effects of anandamide in either normotensive or hypertensive rats; inasmuch as AM251 only led to a more rapid or larger pressor response to anandamide. These data not only confirm our earlier observation that anandamide responses in conscious, normotensive rats are insensitive to AM251 (Gardiner *et al.*, 2002a), but also argue against the possibility of an enhanced involvement of CB_1 receptors as has been suggested in certain hypertensive states (Batkai *et al.*, 2004). Moreover, no consistent cardiovascular effects following infusion of AM251 were detected in either group of rats. Therefore, while these data corroborate and extend previous findings that CB_1 receptor activation modulates regional vascular conductance, heart rate and blood pressure, there is no evidence for significant, basal CB_1 receptor activity (or an anandamide tone) in AII-AVP-induced hypertension. The current study also highlights the importance of molecular targets other than CB_1 receptors, which might provide the primary or parallel signalling pathways, for the cardiovascular effects of anandamide. Future investigations are required to identify the non-CB_1 receptor pathways involved in anandamide responses.

Of particular interest would be the potential role of TRPV1 receptors in the depressor and vasodilator responses to anandamide. Although initial studies have shown that activation of CB_1 receptors mediate the prolonged depressor response (Phase III) of anandamide (Varga *et al.*, 1996; Ledent *et al.*, 1999; Malinowska *et al.*, 2001a), recent evidence suggests that TRPV1 receptors also contribute to the cardiovascular effects of anandamide in certain forms of hypertension, including spontaneously hypertensive rats and rats fed with high-salt diet (Li *et al.*, 2003; Wang *et al.*, 2005). Unlike WIN55212-2, anandamide is known to act as an agonist for both CB_1 receptors and TRPV1 receptors (Zygmunt *et al.*, 1999). In fact, it is established that activation of TRPV1 receptors on perivascular nerves underlie anandamide-induced vasodilatation in mesenteric vascular bed (Jarai *et al.*, 1999; Zygmunt *et al.*, 1999). Thus, it is important that future experiments examine the involvement of TRPV1 receptors in haemodynamic responses to anandamide in acute hypertension. Furthermore, while results from the current study suggest a role for peripheral vasodilatation and CB_1 receptors (in the case for WIN55212-2) in the haemodynamic effects of cannabinoids, they did not elucidate the complex underlying mechanisms for the observed changes in blood pressure and vascular conductances. As discussed above, cardiovascular effects of cannabinoids could result from withdrawal of sympathetic tone, direct vasodilator effects and/or reduction in cardiac contractility (reviews Randall *et al.*, 2004; Pacher *et al.*, 2005a). Additional experiments, for example simultaneous measurements of plasma catecholamine and cardiac contractile function, will help shed light on this matter.

In the course of this study, it was noted that WIN55212-2 and anandamide (at a dose of 3 mg kg^{-1} and only after URB597 treatment) sometimes induced brief behavioural effects, including circling and/or reduced locomotion, in addition to their cardiovascular responses in conscious rats,

which could influence some of the hindquarters responses. Nonetheless, we believe that behavioural effects of the cannabinoids could not explain the haemodynamic responses reported herein. For example, induction of acute hypertension greatly altered the cardiovascular responses, but not the occurrence of behavioural changes, induced by both anandamide and WIN55212-2. It is acknowledged that behavioural effect might be a confounding factor, but it perhaps points to the importance of haemodynamic measurements in the conscious state in evaluating the therapeutic potentials of cannabinoids. Indeed, the pronounced drop in heart rate induced by anandamide in some animals (c.f. Fig. 4; Table 1) also precluded the use of a higher dose of anandamide (>3 mg kg^{-1}) in our experiments in which the animals were fully conscious.

In summary, we have shown that anandamide induced a dose-dependent, delayed hypotension, which was associated with peripheral vasodilatation in conscious, acutely hypertensive rats. The synthetic cannabinoid, WIN55212-2 also caused depressor and enhanced vasodilator effects in acute hypertension. While these data extend previous observations relating to the ability of cannabinoids to lower blood pressure in hypertension, the results clearly show that the mechanisms of action involved depend on the cannabinoids, and it remains unclear whether modulators of the endocannabinoid system are sufficient to normalize cardiovascular variables in hypertension.

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Conflict of interest

None.

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