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# RESEARCH PAPER

# Cardiovascular effects of cannabinoids in conscious spontaneously hypertensive rats

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**Background and purpose:** In anaesthetized spontaneously hypertensive rats (SHR), there is evidence for up-regulation of cannabinoid ($CB_1$) receptors: antagonism of $CB_1$ receptors causes a rise in blood pressure, and administration of the endocannabinoid, anandamide, or inhibition of anandamide degradation causes hypotension. These findings have led to the suggestion that the endocannabinoid system may be a therapeutic target in hypertension. However, since the cardiovascular responses to cannabinoids are substantially influenced by anaesthesia, the purpose of this study was to assess regional haemodynamic responses to cannabinoid receptor stimulation and inhibition in conscious SHR.
**Experimental approach:** Cardiovascular responses to i.v. administration of anandamide, the cannabinoid receptor agonist, WIN 55212-2, and the $CB_1$ receptor antagonist, AM 251, were measured in male SHR, Wistar Kyoto rats and outbred Wistar rats, chronically instrumented for recording renal, mesenteric and hindquarters haemodynamics in the conscious, freely-moving state.
**Key results:** Hypotensive responses to anandamide and WIN 55212-2 only occurred in SHR, but these were relatively modest and not associated with $CB_1$ receptor-mediated vasodilatation. In SHR only, anandamide caused bradycardia, which was inhibited by AM 251. Furthermore, a pressor response to $CB_1$ receptor antagonism occurred only in SHR, but was not associated with vasoconstriction. Moreover, there was some evidence for $CB_1$ receptor-mediated vasoconstrictor actions of anandamide in SHR, which was not seen in the normotensive strains.
**Conclusions and implications:** The results are consistent with activation of $CB_1$ receptors in SHR by endogenous ligands exerting an antihypertensive effect, but the findings do not indicate enhanced $CB_1$ receptor-mediated vasodilator mechanisms in SHR.
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**Keywords:** anandamide; cannabinoids; hypertension; regional haemodynamics; spontaneously hypertensive rats

**Abbreviations:** AM 251, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide; CB, cannabinoid; HDAS, haemodynamics data acquisition system; SHR, spontaneously hypertensive rats; WIN 55212-2, (R)-(+)-(2,3-dihydro-5-methyl-3-[(morphonolinyl)methyl] pyrrolo[1,2,3-de]-1,4-benzoxazin-yl)(1-napthalenyl)methanone mesylate; WKY, Wistar Kyoto rats.

## Introduction

The cardiovascular effects of cannabinoids are of interest, particularly since it has been suggested that targeting the endocannabinoid system may be a novel therapeutic strategy, either by inhibiting their action in hypotensive shock states, or by augmenting their effects in hypertensive conditions (for review, see Pacher *et al*., 2006). In the context of the latter, it has been shown that, in anaesthetized spontaneously hypertensive rats (SHR), there was an enhanced depressor response to administration of the endocannabinoid, anandamide and to an inhibitor of anandamide degradation (Bátkai *et al*., 2004). Furthermore, SHR, but not the normotensive controls, showed a pressor response to cannabinoid ($CB_1$) receptor antagonists, and there was evidence for increased expression of $CB_1$ receptors in the heart and aortic endothelium of the SHR (Bátkai *et al*., 2004). Collectively, these findings indicate an upregulation of the endocannabinoid system in SHR which was exerting a $CB_1$ receptor-mediated antihypertensive effect.

There has been some debate about the underlying haemodynamic changes responsible for the effects of anandamide on blood pressure (for review see Randall *et al*., 2004), and although *in vitro* evidence indicates a vasodilator action of anandamide via multiple mechanisms (O'Sullivan *et al*., 2004), most *in vivo* studies have shown

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that, in the normotensive state, anandamide-induced hypotension is mainly, if not exclusively, due to a fall in cardiac output, rather than a peripheral vascular effect (Bátkai *et al*., 2004, Pacher *et al*., 2005). The very acute, short-lived (1–2 s), hypotensive response to anandamide appears to be due to vagally mediated bradycardia, which does not involve $CB_1$ receptors (Lake *et al*., 1997a, b; Gardiner *et al*., 2002a). In contrast, the slower onset and more sustained, hypotensive effect of anandamide seen in anaesthetized rats is accompanied by modest, $CB_1$ receptor-mediated, bradycardia (Lake *et al*., 1997a, b) and reduced cardiac contractility (Bátkai *et al*., 2004), consistent with *in vitro* evidence for a negative inotropic effect of anandamide, which may (Bonz *et al*., 2003), or may not (Ford *et al*., 2002), involve the $CB_1$ receptor.

However, the study of Bátkai *et al*. (2004) showed that, in the anaesthetized SHR, the enhanced hypotensive effect of anandamide was also, in part, due to a fall in total peripheral resistance, that is attributable to a vasodilator action of the endocannabinoid.

Since the cardiovascular effects of anandamide are known to be influenced by anaesthesia (Lake *et al*., 1997b; Mendizábal and Adler-Graschinsky, 2007), the aim of the present study was to assess the haemodynamic effects of anandamide, and the effects of $CB_1$ receptor antagonism, in conscious, freely moving, SHR. In addition to blood pressure and heart rate, measurements of regional vascular conductance changes were made to determine which vascular beds were contributing to the effects observed. Furthermore, cardiovascular responses to the cannabinoid agonist, WIN 55212-2, were evaluated to determine the extent to which any changes in responsiveness to anandamide were mirrored by changes in response to the synthetic agonist. Due to the long-standing controversy surrounding the appropriate control strain for the SHR (for example, Rapp, 1987; St Lezin *et al*., 1992), two normotensive strains were used for comparison, that is Wistar Kyoto rats (WKY) and outbred Wistar rats.

## Methods

*Animals and surgical preparation*

*Animals*. All procedures were approved by the University of Nottingham Ethical Review Committee, and were performed under UK Home Office Project Licence Authority. Male, spontaneously hypertensive rats (SHR), Wistar rats (Charles River UK) and Wistar Kyoto rats (WKY) (Harlan UK) were housed in a temperature-controlled environment (20–22°C) with a 12 h light/dark cycle (lights on at 0600 hours). Rats were allowed food and water *ad libitum* throughout, and were held within the Biomedical Services Unit in the University of Nottingham for at least a week before commencement of any procedures.

*Surgical preparation*. All surgery was carried out under general anaesthesia (fentanyl and medetomidine, 300 $\mu g\,kg^{-1}$ of each, i.p.), which was reversed by nalbuphine and atipamezole (1 $mg\,kg^{-1}$ of each, s.c.), with nalbuphine also providing analgesia. For some of the later experiments, buprenorphine (0.02 $mg\,kg^{-1}$ s.c.) was used in place of nalbuphine, which was no longer available.

At the first surgical stage, miniaturized Doppler flow probes were sutured around the left renal and superior mesenteric arteries and the distal abdominal aorta (below the level of the ileocaecal artery) for measurement of hindquarters flow.

At least 10 days after probe implantation, and subject to veterinarian checks, rats were again anaesthetized. The Doppler flow probe wires were soldered into a plug (Microtech Inc., Boothwyn, PA, USA), which was mounted into a harness worn by the rat. Three separate catheters were inserted into the right jugular vein to allow drug administration, and a single catheter was inserted into the distal abdominal aorta via the caudal artery, enabling arterial blood pressure and heart rate measurement. Animals were left to recover for 24 h before experiments began.

At the time of experimentation, male Wistar rats weighed between 350 and 450 g, whereas SHR (~20 weeks old) and WKY weighed approximately 300 g. In the SHR, protocols for the administration of anandamide and WIN 55212-2 were carried out in separate groups of animals. In WKY and Wistar rats, however, a combined anandamide and WIN 55212-2 protocol was used.

*Experimental protocols*

*Spontaneously hypertensive rats*. In one group of SHR ($n=10$), on the first experimental day, after a period of baseline recording, the vehicle for anandamide (Tocrisolve, 0.1 ml i.v.) was administered followed, at least 60 min later, by anandamide (3 $mg\,kg^{-1}$ i.v.). On the second experimental day, AM 251 (3 $mg\,kg^{-1}$ i.v. infused over 30 min at 2 $ml\,h^{-1}$; Gardiner *et al*., 2002a, b) was administered and 30 min after the end of the AM 251 infusion, animals were given anandamide (3 $mg\,kg^{-1}$).

In a second group of SHR ($n=8$), on the first experimental day, after a period of baseline recording, the vehicle for WIN 55212-2 (saline containing 5% propylene glycol and 2% Tween-80) was administered (0.1 ml i.v.), followed by WIN 55212-2 (150 $\mu g\,kg^{-1}$) at least 120 min later. On the second experimental day, AM 251 (3 $mg\,kg^{-1}$ i.v. infused over 30 min at 2 $ml\,h^{-1}$) was administered and 30 min after the end of the AM 251 infusion, animals were given WIN 55212-2 (150 $\mu g\,kg^{-1}$).

*Wistar Kyoto rats*. One group of WKY ($n=12$) was used. On day 1, animals were given anandamide (3 $mg\,kg^{-1}$) and the vehicle (0.1 ml) in random order, separated by at least 180 min. On day 2, the same animals were given the vehicle for WIN 55212-2 (see above) followed by WIN 55212-2 (150 $\mu g\,kg^{-1}$) at least 120 min later. On day 3, in a subgroup of seven animals, AM 251 was administered (3 $mg\,kg^{-1}$ as above) followed by WIN 55212-2 (150 $\mu g\,kg^{-1}$) and anandamide (3 $mg\,kg^{-1}$), given in random order, separated by at least 60 min.

*Wistar rats*. One group of Wistar rats was used ($n=10$). On day 1, after a period of baseline recording, the vehicle for WIN 55212-2 was administered (as above) followed by WIN 55212-2 (150 $\mu g\,kg^{-1}$) at least 120 min later. After a further period of at least 120 min, when all the cardiovascular effects of WIN 55212-2 had waned, anandamide (3 $mg\,kg^{-1}$) was

given. On day 2, AM 251 ($3\,mg\,kg^{-1}$ as above) was administered, and 30 min after the end of infusion, anandamide ($3\,mg\,kg^{-1}$) was administered followed by WIN 55212-2 ($150\,\mu g\,kg^{-1}$) 60 min later. In other experiments (Gardiner *et al*., 2002b) we have shown that the dose of AM 251 used here is effective for at least 5 h.

*Cardiovascular measurements*

*Data acquisition*. All data were recorded by a customized data capture system (Haemodynamics Data Acquisition System (HDAS), University of Limburg, Maastricht, Netherlands). Arterial catheters were attached via a fluid-filled (degassed water) pressure transducer (Gould, type 4-442) with a modified, low volume displacement dome, to a Gould transducer amplifier (model 13-4615-50) and into HDAS. The Doppler flow probe leads were then connected via a Doppler flowmeter (Crystal Biotech VF-1 Mainframe fitted with high velocity (HVPD-20) modules). These data were sampled by HDAS every 2ms, averaged every cardiac cycle and then stored to disc at 5-sec intervals thus enabling recording of heart rate, blood pressure and processed Doppler shift signals. The initial response to anandamide, which occurs within 1–2 cardiac cycles, is highly variable at the dose used (Gardiner *et al*., 2002a) and is generally agreed not to involve $CB_1$ receptors (Lake *et al*., 1997b), and was not analysed in this study.

*Statistical analysis*

Offline analysis of averaged values from experimenter-selected time intervals was conducted using Datview software (University of Limburg, Maastricht, Netherlands). Values were then exported into custom-designed software (Biomed, University of Nottingham) for statistical analysis. The Friedman and Quade tests (non-parametric versions of ANOVA) were used for within-group analysis, Wilcoxon tests were conducted for comparing paired datasets, and Mann–Whitney tests were used for between-group comparisons, where $P<0.05$ was taken as significant. Multiple comparisons of integrated areas between strains and treatments were calculated using a Kruskal–Wallis test.

*Drugs*

Fentanyl citrate was from Janssen–Cilag (High-Wycombe, UK); medetomidine hydrochloride (Domitor) and atipamezole hydrochloride (Antisedan) were from Pfizer (Kent, UK); nalbuphine hydrochloride (Nubain) was from Bristol-Myers-Squibb (Hounslow, UK); buprenorphine (Vetergesic) was from Alstoe Animal Health (York, UK). Anandamide, Tocrisolve, WIN 55212-2, ((*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-1naphthalenylmethanone mesylate) and AM 251 (*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrozole-3-carboxamide) were from Tocris (Bristol, UK), with anandamide supplied dissolved in Tocrisolve. AM 251 and WIN 55212-2 were dissolved in saline with 5% propylene glycol (Sigma UK) and 2% Tween-80 (BDH). AM 251 was infused ($2\,ml\,h^{-1}$) over 30 min, and all other drugs were administered as bolus intravenous injections given in a volume of approximately 0.1 ml.

## Results

*Cardiovascular responses to anandamide in the absence and presence of AM 251*

Resting cardiovascular variables before administration of anandamide are shown in Table 1. In SHR, resting heart rates and blood pressures were significantly higher, and vascular conductances were generally lower ($P<0.05$ for renal and mesenteric vs WKY; $P<0.05$ for renal and hindquarters vs Wistar rats) than in either strain of control rat. Wistar rats had lower blood pressures and lower renal and mesenteric vascular conductances than WKY (Table 1).

As described above (see Methods), the initial, rapid and very transient response to anandamide, which tends to be highly variable in conscious animals at the dose used (Gardiner *et al*., 2002a), was not analysed in this study. In WKY and in Wistar rats, the cardiovascular effects of anandamide were modest, the only significant ($P<0.05$ Friedman's test) changes directly associated with anandamide being a small rise in blood pressure in Wistars, and hindquarters vasodilatation in WKY (Figure 1). In contrast, in SHR, anandamide caused pronounced, sustained bradycardia, together with a rise followed by a fall in blood pressure and renal and mesenteric vasoconstriction, followed by some tendency for vasodilatation in all three vascular beds, albeit only significant in the renal vascular bed (Figure 1). The integrated (0–30 min) bradycardic response to anandamide in SHR ($-700\pm225$ beats) was significantly ($P<0.05$) greater than in WKY ($-86\pm31$ beats) and Wistar

**Table 1** Resting cardiovascular variables

| | *Before anandamide* | | | *Before WIN 55212-2* | | |
|---|---|---|---|---|---|---|
| | *Wistar (n = 10)* | *WKY (n = 12)* | *SHR (n = 14)* | *Wistar (n = 10)* | *WKY (n = 11)* | *SHR (n = 8)* |
| Heart rate (beats $min^{-1}$) | $319\pm6$ | $325\pm6$ | $353\pm10$*# | $327\pm6$ | $309\pm4$* | $330\pm11$ |
| Mean arterial BP (mm Hg) | $111\pm2$ | $120\pm3$* | $176\pm5$*# | $113\pm3$ | $122\pm2$* | $175\pm4$*# |
| Renal VC (kHz mm $Hg^{-1}10^3$) | $58\pm7$ | $87\pm7$* | $40\pm6$*# | $59\pm9$ | $80\pm5$* | $41\pm4$*# |
| Mesenteric VC (kHz mm $Hg^{-1}10^3$) | $57\pm6$ | $83\pm6$* | $41\pm4$# | $59\pm6$ | $83\pm7$* | $46\pm6$# |
| Hindquarters VC (kHz mm $Hg^{-1}10^3$) | $39\pm4$ | $30\pm3$ | $25\pm2$* | $40\pm4$ | $25\pm1$* | $27\pm3$* |

Abbreviations: SHR, spontaneously hypertensive rats; VC, vascular conductance; WKY, Wistar Kyoto rats.
Values are mean±s.e.means. VC is calculated as Doppler shift (kHz) divided by mean arterial blood pressure (mm Hg).
*$P<0.05$ vs Wistar, # $P<0.05$ vs WKY (Kruskal–Wallis).

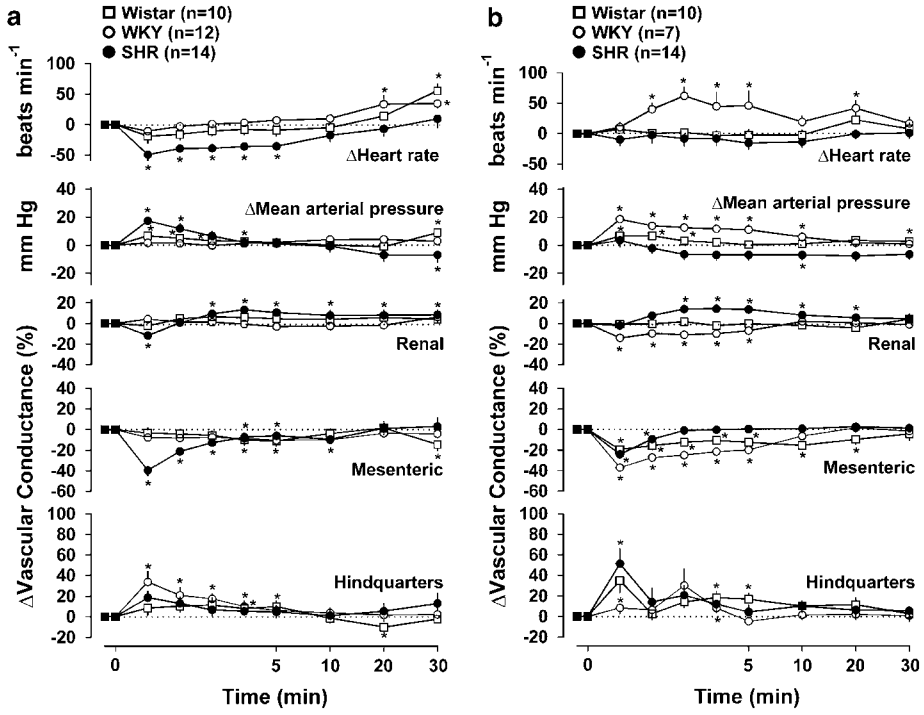


**Figure 1** Regional haemodynamic responses to anandamide (3 mg kg$^{-1}$ i.v.) in conscious Wistar rats, WKY and spontaneously hypertensive rats, in the absence (**a**) and presence (**b**) of AM 251 (3 mg kg$^{-1}$ i.v.). Values are mean and vertical bars show s.e.m. *$P<0.05$ vs baseline (Friedman's test). Statistical comparisons of integrated responses are given in the text.

rats ($-205\pm80$ beats). Moreover, although the integrated pressor responses were not different between the three strains, the integrated depressor response was greater ($P<0.05$) in SHR ($-222\pm67$ mm Hg min) than in WKY ($-36\pm14$ mm Hg min) and Wistar rats ($-27\pm10$ mm Hg min). However, there were no significant between-strain differences in the integrated changes in vascular conductance in response to anandamide (either increases or decreases). Administration of the vehicle for anandamide (Tocrisolve) to WKY caused no significant changes (Friedman's test) in any measured cardiovascular variable (data not shown). In SHR, following administration of Tocrisolve, there was a small reduction in heart rate which was only significant 1 min following the vehicle administration ($-15\pm4$ beats min$^{-1}$, $P<0.05$, Friedman's test), and the integrated (0–30 min) bradycardic response to anandamide in SHR ($-700\pm225$ beats) was significantly greater than the integrated change following the vehicle in that strain ($-239\pm115$ beats). There were no significant changes in any other measured cardiovascular variable following vehicle administration to SHR (data not shown).

Administration of AM 251 had no consistent cardiovascular effects in either Wistar rats or in WKY, but in SHR, there was a significant increase in blood pressure between 20 and 60 min after the onset of AM 251 administration ($+12\pm3$ mm Hg at 20 min, $+14\pm3$ mm Hg at 40 min), but this pressor effect was not accompanied by significant changes in heart rate or vascular conductance in any monitored vascular bed (at 20 min: heart rate $+13\pm9$ beats min$^{-1}$, renal vascular conductance $-5\pm3\%$, mesenteric vascular conductance $-8\pm3\%$, hindquarters vascular conductance $+4\pm4\%$; at 40 min: heart rate $+19\pm10$ beats min$^{-1}$, renal vascular conductance $-1\pm3\%$, mesenteric vascular conductance $-9\pm4\%$, hindquarters vascular conductance $-4\pm3\%$).

In the presence of AM 251, the cardiovascular effects of anandamide in Wistar rats were still modest, although there was some mesenteric vasoconstriction and hindquarters vasodilatation which were not seen in the absence of AM 251 (Figure 1b). Paradoxically, the cardiovascular effects of anandamide in WKY in the presence of AM 251 were more substantial than in the absence of AM 251, comprising an early rise in blood pressure and renal and mesenteric vasoconstriction and a slower onset tachycardia, none of which occurred in the absence of AM 251 (Figure 1b). In contrast, in SHR, AM 251 abolished the bradycardic, pressor and renal vasoconstrictor responses to anandamide and attenuated the mesenteric vasoconstriction. Under those conditions, anandamide still caused some fall in blood pressure and there was renal and hindquarters vasodilatation (Figure 1b).

*Cardiovascular responses to WIN 55212-2 in the absence and presence of AM 251*

In SHR, before administration of WIN 55212-2, resting blood pressures were significantly higher than in either control strain of rat, and vascular conductances were lower ($P<0.05$ for renal and hindquarters vs Wistar rats; $P<0.05$ for renal and mesenteric vs WKY) (Table 1). In Wistar rats, blood pressures and renal and mesenteric vascular conductances were lower, and heart rate and hindquarters vascular conductance were higher, than in WKY (Table 1).

Administration of WIN 55212-2 (150 $\mu$g kg$^{-1}$) caused rises in blood pressure, falls in renal and mesenteric vascular

conductances and rises in hindquarters vascular conductance in all three strains of rat (Figure 2a), and the integrated (0–10 min) pressor and vascular conductance changes were not significantly different between the strains. In SHR and WKY there was no significant change in heart rate, but in Wistar rats there was bradycardia lasting up to 10 min after WIN 55212-2 administration (Figure 2a). In SHR only, the rise in blood pressure was followed by a fall ($-22\pm5$ mm Hg at 30 min), accompanied by vasodilatation (significant in the renal and mesenteric vascular beds) (Figure 2a). The vehicle for WIN 55212-2 caused no changes in heart rate in either SHR or WKY, and, although it caused a small fall in heart rate in Wistar rats ($P<0.05$ at 4 and 5 min, $-17\pm7$ and $-18\pm7$ beats min$^{-1}$, respectively), the integrated change in heart rate caused by WIN-55212-2 in that strain was significantly greater than the effect of vehicle. Blood pressure was not significantly affected by the vehicle in either WKY or Wistar rats, and in SHR, although there was a small significant increase in blood pressure following the vehicle ($P<0.05$ between 0 and 5 min), the magnitude of change ($+7\pm3$ mm Hg at 1 min) was markedly less than the corresponding change following WIN 55212-2 ($+33\pm5$ mm Hg), and there was no subsequent hypotensive response to the vehicle ($+5\pm4$ mm Hg at 30 min). Renal vascular conductance was not affected by vehicle administration, mesenteric vascular conductance fell transiently in SHR and WKY (maximum change $-14\pm5$ and $-8\pm2$% at 1 min respectively), and hindquarters vascular conductance rose transiently in WKY ($+17\pm7$% at 1 min), but the magnitude of change in all variables was substantially and significantly smaller than the effect of WIN 55212-2.

In the presence of AM 251, the pressor and renal vasoconstrictor responses to WIN 55212-2 were abolished in all three strains of rat. The integrated (0–10 min) fall in mesenteric vascular conductance in response to WIN 55212-2 was significantly reduced by AM 251 in WKY (before $=-339\pm28$% min, after $=-102\pm14$% min) and Wistar rats (before $=-178\pm36$% min, after $=-66\pm22$% min), and, although the response was reduced in SHR (before $=-264\pm68$% min, after $=-155\pm47$% min) the difference was not significant. Similarly, the integrated (0–10 min) hindquarters vasodilator response to WIN 55212-2 was reduced by AM 251 in all three strains (WKY before $=+288\pm56$% min, after $=+81\pm26$% min; Wistar rats before $=+322\pm54$% min, after $=+158\pm49$% min; SHR before $=+324\pm54$% min, after $=+233\pm55$% min), but the effect was not significant in SHR. The delayed fall in blood pressure, seen in SHR in response to WIN 55212-2 in the absence of AM 251, was still present following AM 251 administration ($-22\pm5$ mm Hg at 30 min); indeed a small fall in blood pressure also occurred in Wistar rats under these conditions, but the only significant vasodilatation was in the renal vascular bed in SHR (Figure 2b).

## Discussion and conclusions

Against the background of evidence for increased involvement of endocannabinoids in cardiovascular regulation in anaesthetized SHR (Bátkai *et al*., 2004), the specific aim of the present study was to determine the regional haemodynamic effects of the endocannabinoid, anandamide, the $CB_1$

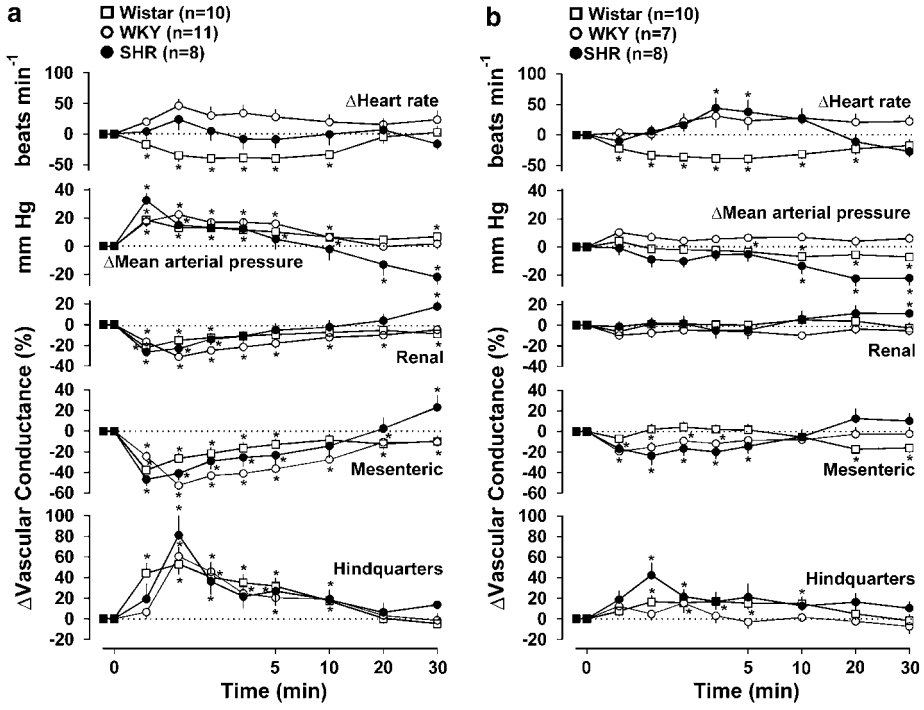


**Figure 2** Regional haemodynamic responses to WIN 55212-2 (150 $\mu$g kg$^{-1}$ i.v.) in conscious Wistar rats, WKY and spontaneously hypertensive rats, in the absence (**a**) and presence (**b**) of AM 251 (3 mg kg$^{-1}$ i.v.). Values are mean and vertical bars show s.e.m. *$P<0.05$ vs baseline (Friedman's test). Statistical comparisons of integrated responses are given in the text.

receptor antagonist, AM 251 and the synthetic cannabinoid agonist, WIN 55212-2, in conscious SHR, and compare them with the responses in two normotensive Wistar strains, that is WKY and outbred Wistar rats.

In normotensive rats, the cardiovascular actions of anandamide are complex and influenced by the presence of anaesthesia (for reviews see Randall *et al*., 2004; Mendizábal and Adler-Graschinsky, 2007). In conscious Sprague–Dawley rats, within 1–2 min after administration of anandamide (that is, after the very rapid, short-lived changes), the cardiovascular effects are modest, comprising a small rise in blood pressure and a bradycardia (Stein *et al*., 1996; Lake *et al*., 1997a, b; Gardiner *et al*., 2002a; Wheal *et al*., 2007a), with some renal and mesenteric vasoconstriction and hindquarters vasodilatation (Gardiner *et al*., 2002a; Wheal *et al*., 2007a). In the normotensive rat strains used in the present study, the cardiovascular effects of anandamide were qualitatively similar to those described previously (excluding the rapid initial effects not measured here), but smaller than observed in response to the same dose in Sprague–Dawley rats under the same conditions (Gardiner *et al*., 2002a; Wheal *et al*., 2007a).

Lake *et al*. (1997b) showed that the pressor response to anandamide in conscious Sprague–Dawley rats was enhanced by the $CB_1$ receptor antagonist, SR 141716A, and, although we previously found no effect of AM 251 on responses to anandamide in Sprague–Dawley rats (Gardiner *et al*., 2002a), in the present study we observed some AM 251-induced enhancement of pressor and vasoconstrictor responses to anandamide in the Wistar rats and, particularly, in WKY. These findings are consistent with there being a covert $CB_1$ receptor-mediated vasodilator effect of anandamide in normotensive rats that is not normally seen, because the pressor and vasoconstrictor effects of anandamide predominate. The mechanisms underlying the latter are complex, but are generally agreed not to involve $CB_1$ receptors (Lake *et al*., 1997a, b; Kwolek *et al*., 2005). Kwolek *et al*. (2005) proposed a central mechanism for the anandamide-induced pressor response involving $\beta$-adrenoceptors and NMDA receptors located in the medulla oblongata. Although the evidence for an involvement of sympathetic activation is not strong (Kwolek *et al*., 2005), our finding of a tachycardic effect of anandamide in WKY in the presence of AM 251, would be consistent with it causing sympathetic activation, either by peripheral or central mechanisms. Alternatively, it has been shown in isolated atria, that anandamide caused an increase in cardiac contractility in the presence of $CB_1$ receptor antagonism and a decrease in contractility in the presence of $CB_2$ receptor antagonism, suggesting the presence of functional myocardial $CB_1$ and $CB_2$ receptors causing cardio-depression and cardio-excitation, respectively (Sterin-Borda *et al*., 2005). The tachycardic effect of anandamide in WKY in the presence of AM 251 may, therefore, be a manifestation of $CB_2$ receptor-mediated cardioexcitation.

To our knowledge, only three studies have reported cardiovascular effects of anandamide in SHR previously (Lake *et al*., 1997b; Li *et al*., 2003; Bátkai *et al*., 2004), and only one of those was in conscious animals (Lake *et al*., 1997b). In that study, after the initial, rapid hypotensive and bradycardic effects of anandamide, there was a short-lived (1–2 min) rise in blood pressure. The present findings corroborate and extend that observation, showing that the transient pressor response to anandamide in SHR, which was more marked than in the control strains, was accompanied by renal and mesenteric vasoconstriction. Furthermore, and in contrast to the findings in the normotensive animals, we found these effects were attenuated by AM 251, indicating $CB_1$ receptor-mediated vasoconstrictor effects in SHR.

The vast majority of evidence indicates that anandamide is a vasorelaxant in isolated arterial vessels from normotensive rats (see Randall *et al*., 2004) and, in our experience, SHR (Wheal *et al*., 2007b). To our knowledge, there is only one report of anandamide causing vasoconstriction, and that was in rat mesenteric arteries in calcium-free buffer (White and Hiley, 1998). Therefore, it is most likely that the pressor and vasoconstrictor effects are indirect. As indicated above, studies by Kwolek *et al*. (2005) have gone some way to identifying the mechanisms involved in the pressor response to anandamide in anaesthetized normotensive rats, but it seems that the mechanisms may differ in SHR, since the effect of AM 251 was the opposite in normotensive rats (that is, augmentation) than in SHR (that is, attenuation). Our finding that the pressor response was reduced by AM 251 in SHR is in contrast to the reports of Lake *et al*. (1997b), who showed that the pressor response was unchanged by SR 141716A in that strain, although they saw an enhancement by SR 141716A in their normotensive animals.

Lake *et al*. (1997b) described, in addition, a delayed hypotensive response to anandamide (significant between 10 and 45 min) in conscious SHR, and other studies have reported exaggerated hypotensive responses in anaesthetized SHR compared to normotensive rats (Lake *et al*., 1997b; Li *et al*., 2003; Bátkai *et al*., 2004). Li *et al*. (2003) found enhanced hypotensive responses to methanandamide (the stable analogue of anandamide) in SHR relative to WKY and provided evidence for an involvement of vanilloid receptors in addition to $CB_1$ receptors, whereas Bátkai *et al*. (2004) found no effect of the vanilloid receptor antagonist, capsazepine, on the enhanced hypotensive response to anandamide in SHR and Lake *et al*. (1997b) showed that the $CB_1$ receptor antagonist, SR 141716A, abolished the depressor response. In the present study we observed a small (about 7 mm Hg), delayed hypotensive response to anandamide in conscious SHR, which was accompanied by some evidence for vasodilatation, albeit only significant in the renal vascular bed. The magnitude of effect was considerably less than reported by Lake *et al*. (1997b), who showed a fall of about 25 mm Hg at 20 min after a similar dose of anandamide (4 mg $kg^{-1}$), but their data are not inconsistent with the findings of Bátkai *et al*. (2004), who showed a more marked (about 50 mm Hg) hypotensive response to a higher dose of anandamide (10 mg $kg^{-1}$) in anaesthetized SHR which was, in part, due to vasodilatation. In the present study, neither the fall in blood pressure nor the renal vasodilatation were inhibited by AM 251, indicating that they may not involve $CB_1$ receptors. However, the bradycardic response to anandamide which was marked in the SHR, was inhibited by AM 251, suggesting a $CB_1$ receptor-mediated cardiac effect in that strain of rats, consistent with the results of Lake *et al*.

(1997a, b), and consistent with evidence for increased expression of $CB_1$ receptors in the myocardium of SHR (Bátkai *et al*., 2004). It is feasible that the $CB_1$ receptor-mediated bradycardic effect of anandamide was due to a direct (Bonz *et al*., 2003; Sterin-Borda *et al*., 2005) and/or indirect action, such as inhibition of noradrenaline release (Molderings *et al*., 1999). Additionally, our results showed a modest pressor response to AM 251 in SHR, which was not accompanied by vasoconstriction or a change in heart rate suggesting an underlying change in cardiac function. These data with AM 251 support the findings of Bátkai *et al*. (2004), although the magnitude of change in our conscious rats (about 14 mm Hg) was substantially less than the effect reported by Bátkai *et al*. (2004) with SR 141716A in anaesthetized SHR (about 30 mm Hg) and with AM 251 in anaesthetized rats made hypertensive by infusion of angiotensin II (about 50 mm Hg).

If some of the present findings with anandamide and AM 251 are interpreted as indicating upregulation of $CB_1$ receptor-mediated cardiovascular effects in SHR, then exaggerated responses to a synthetic $CB_1$ receptor agonist would have been expected. However, we have shown previously that although the pressor and vascular responses to WIN 55212-2 in conscious rats involve $CB_1$ receptors, the latter are involved indirectly via activation of sympathoadrenal mechanisms (Gardiner *et al*., 2001, 2002b). Thus, the present findings indicate those processes are not altered in SHR. However, following the pressor response to WIN 55212-2 in SHR, there was a delayed hypotension, accompanied by vasodilatation in renal and mesenteric vascular beds, and although the hypotension and renal vasodilatation were not inhibited by AM 251, the mesenteric vasodilatation was abolished.

An unexpected effect of WIN 55212-2 was a bradycardia in Wistar rats, which was not sensitive to AM 251, and not attributable to a baroreceptor reflex, since it was still present when the pressor effect was abolished. This may reflect a non-cannabinoid action of WIN 55212-2, for example, direct inhibition of calcium channels (Shen and Thayer, 1998; Ho and Hiley, 2003), but it is not clear why it is not seen in other normotensive strains either in this study (WKY) or previous studies in Sprague–Dawley rats (Gardiner *et al*., 2002b; Wheal *et al*., 2007a).

Collectively, the results show some differences in the responses to anandamide and WIN 55212-2 in SHR compared to normotensive rats, but the differences are not convincingly explained by upregulation of $CB_1$ receptor-mediated vasodilator mechanisms. We have shown a small hypotensive response to anandamide, and a more marked hypotensive response to WIN 55212-2 in SHR, neither of which are susceptible to inhibition by AM 251, and neither of which are likely to be wholly attributable to vasodilatation, suggesting a negative effect on cardiac function (Ford *et al*., 2002), rather than a vasodilator effect of vanilloid receptor stimulation (Li *et al*., 2003). Interestingly, we have recently shown that in rats made hypertensive by nitric oxide synthase inhibition, there is no evidence for a delayed hypotensive effect of anandamide and/or enhanced vasodilator actions, and no evidence for a pressor response to $CB_1$ receptor antagonism (Wheal *et al*., 2007a), indicating that the phenomenon is not common to all hypertensive states.

Thus, while there is persuasive evidence for vasodilator effects of cannabinoids *in vitro*, and some evidence for enhanced vasodilator effects of cannabinoids in hypertensive states under anaesthesia, the outcome of the present study in conscious rats makes it unlikely that $CB_1$ receptor-mediated vasodilator effects of cannabinoids could be exploited as a potential target for antihypertensive therapy.

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## Conflict of interest

The authors state no conflict of interest.

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