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Influence of the CB_1 receptor antagonist, AM 251, on the regional haemodynamic effects of WIN-55212-2 or HU 210 in conscious rats

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1 In conscious, freely-moving, male, Sprague-Dawley rats, the regional haemodynamic responses to the synthetic cannabinoids, WIN-55212-2 and HU 210, were compared. The possible involvement of cannabinoid, CB_1-receptors, or β_2-adrenoceptors in the responses to WIN-55212-2 and HU 210 were investigated using the CB_1-receptor antagonist, AM 251, or the β_2-adrenoceptor antagonist, ICI 118551, respectively.

2 Both WIN-55212-2 (150 μg kg^{-1}) and HU 210 (100 μg kg^{-1}) had pressor, renal, and mesenteric vasoconstrictor and hindquarters vasodilator actions, although the effects of HU 210 were much more sustained than those of WIN-55212-2. Lower doses of the cannabinoids (WIN-55212-2, 50 μg kg^{-1}, HU 210, 10 μg kg^{-1}) had less consistent actions.

3 All the significant cardiovascular effects of WIN-55212-2 and HU 210 were antagonized by pretreatment with AM 251 (3 mg kg^{-1}). Furthermore, pretreatment with the β_2-adrenoceptor antagonist, ICI 118551, inhibited the hindquarters vasodilator effects of WIN-55212-2 and of HU 210.

4 On the basis of the present findings, and our earlier work, it is suggested that, in conscious rats, the pressor and vasoconstrictor effects of HU 210 and WIN-55212-2 involve cannabinoid-receptor-mediated increases in sympathetic activity. The accompanying hindquarters vasodilator actions of these agonists are cannabinoid receptor-mediated and appear to involve β_2-adrenoceptors.
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Introduction

Previously (Gardiner *et al*., 2001), we have drawn attention to the complexity of the cardiovascular effects of cannabinoids as described in the literature, and have suggested that this might reflect variations in experimental conditions.

Considering the *in vivo* studies, it appears that the state of anaesthesia influences the cardiovascular response to synthetic cannabinoids, since we have recently reported that WIN-55212-2, which has been shown to have marked hypotensive effects in anaesthetized rats (Lake *et al*., 1997), has dose-dependent pressor and renal and mesenteric vasoconstrictor effects in conscious rats, with hindquarters vasodilator actions being seen only with the higher doses of the cannabinoid (Gardiner *et al*., 2001).

In our previous study, we made no formal assessment of the involvement of cannabinoid receptors in the vasoconstrictor or vasodilator responses to WIN-55212-2. Thus, the first aim of the present study was to determine the effects of the cannabinoid, CB_1-receptor antagonist, AM 251 (Gatley *et al*., 1996, 1997) on the cardiovascular responses to WIN-55212-2 in conscious rats. We considered this an important experiment since, unlike SR 141716A, AM 251 is commercially available and yet there are no published *in vivo* data on the cardiovascular effects of AM 251, either on baseline haemodynamic status, or on responses to cannabinoids. In addition, since we (Gardiner *et al*., 2001) have hypothesized that the hindquarters vasodilator action of WIN-55212-2 could be mediated by β_2-adrenoceptors, our second aim was to assess the effects of the β_2-adrenoceptor antagonist, ICI 118551 (Bilski *et al*., 1983), on responses to WIN-55212-2.

In anaesthetized rats, other cannabinoid agonists, such as HU 210, which differ structurally from WIN-55212-2 (for review see Pertwee, 1997), have also been reported to cause CB_1-receptor-mediated hypotension, and this has been taken as a sign of vasodilatation (e.g., Lake *et al*., 1997). However, it is clear from our studies with WIN-55212-2, that it is only by monitoring regional haemodynamics that one can get a proper picture of the processes underlying any effects substances, such as cannabinoids, have on systemic arterial blood pressure. Very recently, some work has been published which indicates a complex regional haemodynamic profile associated with administration of HU 210 in urethane-anaesthetized rats (Wagner *et al*., 2001). However, because of the technique they used, Wagner *et al*. (2001) were restricted to making measurements at a single point in time following HU 210 administration. We know of only one report of the cardiovascular effects of HU 210 in conscious

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rats (Vidrio *et al*., 1996), and in that study, the cannabinoid was administered i.p., and measurements were restricted to blood pressure and heart rate, monitored indirectly. Therefore, the final aim of the present study was to make continuous measurements of the regional haemodynamic effects of HU 210 in conscious rats, to ascertain whether or not they resembled those of WIN-55212-2, and to determine the effects of cannabinoid receptor antagonism (with AM 251), or β_2-adrenoceptor antagonism (with ICI 118551), thereupon.

Some of the results have been presented to the British Pharmacological Society (Gardiner *et al*., 2002a,b).

Methods

All experiments were carried out on male, Sprague-Dawley rats (Charles River, U.K.), weighing between 420 and 470 g at the time of study. Animals 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 and water throughout. The procedures were approved by the University of Nottingham Ethical Review Committee, and were performed under Home Office Project Licence authority.

Surgical preparation

Surgery was performed in two stages, under general anaesthesia (fentanyl and meditomidine, 300 μg kg^{-1} of each, i.p., reversed with nalbuphine and atipamezole 1 mg kg^{-1} of each, s.c.). The first stage involved implantation of miniaturized pulsed Doppler flow probes around the left renal artery, the superior mesenteric artery and the distal abdominal aorta (below the level of the ileocaecal artery, to monitor flow to the hindquarters). The second stage involved placement of catheters in the distal abdominal aorta (*via* the caudal artery) to monitor arterial blood pressure and heart rate, and in the right jugular vein for drug administrations. The two surgical stages were separated by at least 10 days and, prior to the second stage, the fitness of all animals was certified by the named veterinary surgeon.

Experiments began 24 h after catheterization, when the animals were fully conscious, freely moving, and with access to food and water *ad libitum*.

Cardiovascular recordings

Continuous recordings of cardiovascular variables were made using a customized, computer-based system (Haemodynamics Data Acquisition System (HDAS), University of Limburg, Maastricht, Netherlands) connected to the transducer amplifier (Gould model 13-4615-50) and the Doppler flowmeter (Crystal Biotech VF-1 mainframe (pulse repetition frequency 125 kHz) fitted with high velocity (HVPD-20) modules). Raw data were sampled by HDAS every 2 ms, averaged every cardiac cycle and stored to disc at 5 s intervals. Data were analysed offline using software (Datview, University of Limburg, Maastricht, Netherlands) which interfaced with HDAS.

Cardiovascular responses to WIN-55212-2 in the absence or presence of AM 251.

Animals ($n=8$) were given i.v. bolus doses of WIN-55212-2 (50 and 150 μg kg^{-1}) at 45 min intervals, starting 30 min after the end of administration of the cannabinoid antagonist AM 251 (3 mg kg^{-1} infused i.v. over 30 min at 2 ml h^{-1}), or the vehicle for AM 251 (saline containing 5% propylene glycol and 2% Tween 80). The low dose of WIN-55212-2 was always given first. Animals were randomized to receive vehicle or AM 251 on the first day and the other treatment on the third day, with the intervening day being allowed to ensure adequate washout of any active treatment.

Cardiovascular responses to HU 210 in the absence or presence of AM 251

The cardiovascular effects of HU 210 develop slowly and are long-lasting (see Results). Therefore, in one group of animals ($n=8$), we administered HU 210 (10 μg kg^{-1}) on the first experimental day, vehicle (saline containing 5% propylene glycol and 2% Tween 80) on the second day, and HU 210 (100 μg kg^{-1}) on the third day. Cardiovascular variables were recorded continuously for 5 h on each day.

In separate groups of animals, responses to HU 210 (100 μg kg^{-1}) were assessed 45 min after administration of AM 251 (3 mg kg^{-1} as above, $n=7$) or vehicle (as above, $n=6$), and monitored for 5 h. As a time control, a separate group of animals ($n=7$) was given AM 251 followed by the vehicle for HU 210 (doses and timings as above).

The experiments involving administration of WIN-55212-2 showed clear-cut antagonism of its cardiovascular effects by AM 251 (see below). Therefore, at the end of the 5 h recording period following HU 210 administration, all animals were given a single dose of WIN-55212-2 (150 μg kg^{-1}) to check for adequacy of blockade by AM 251.

Cardiovascular responses to WIN-55212-2 in the absence or presence of ICI 118551

In preliminary experiments ($n=2$) we confirmed the reproducibility of the responses to WIN-55212-2 (150 μg kg^{-1}) in the absence of ICI 118551 (see Results).

In the main experiment, rats ($n=7$) were given WIN-55212-2 (150 μg kg^{-1}) before and 90 min after administration of ICI 118551 (0.2 mg kg^{-1} bolus, 0.1 mg kg^{-1} h^{-1} infusion) (Gardiner & Bennett, 1988; Gardiner *et al*., 1992). To check the effectiveness of this dose of ICI 118551 in the present conditions, four animals were given a 3 min infusion of salbutamol (600 ng kg^{-1} min^{-1}) before and 90 min after the onset of administration of ICI 118551. Before ICI 118551, 3 min after the onset of infusion of salbutamol, there was an increase in heart rate ($+86\pm18$ beats min^{-1}) and hindquarters vascular conductance ($+134\pm24\%$), a fall in blood pressure (-9 ± 4 mmHg) and no change in renal or mesenteric vascular conductances. In the presence of ICI 118551, salbutamol caused no change in heart rate or blood pressure, and the increase in hindquarters vascular conductance ($+22\pm3\%$) was markedly inhibited.

Cardiovascular responses to HU 210 in the presence of ICI 118551

Rats ($n=8$) were given HU 210 (100 μg kg^{-1}) 90 min after the start of administration of ICI 118551 (0.2 mg kg^{-1} bolus, 0.1 mg kg^{-1} h^{-1} infusion).

Data analysis

Within-group analyses were by non-parametric analysis of variance (Friedman's test; Theodorsson-Norheim, 1987).

All between-group comparisons were confined to the integrated responses to the cannabinoids, measured as the areas under or over the time curves (0–5 min for WIN-55212-2, 0–300 min for HU 210). Between-group analyses were by the Wilcoxon or Mann–Whitney *U*-test (two groups), or the Kruskal–Wallis test with Dunn's post-test (more than two groups). A P value $\leqslant 0.05$ was taken as significant. Statistical tests were performed using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA, U.S.A.; licensed to School of Biomedical Sciences, University of Nottingham, U.K.).

Drugs

Fentanyl citrate was obtained from Martindale; meditomidine hydrochloride (Domitor) and atipamezole hydrochloride (Antisedan) were obtained from Pfizer; nalbuphine hydrochloride (Nubain) was obtained from Du Pont: WIN-55212-2, ((R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate); AM 251, (N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide); HU 210 ((6aR)-*trans*-3-(1,1-Dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol) and ICI 118551 hydrochloride were obtained from Tocris (U.K.). Solutions of AM 251, HU 210 and WIN-55212-2 were prepared fresh daily in sterile saline containing 5% propylene glycol (Sigma) and 2% Tween 80 (B.D.H.). ICI 118551 was dissolved in sterile water. Bolus injections were given in a volume of 0.1 ml; AM 251 was infused at a rate of 2 ml h^{-1} for 30 min. There were no cardiovascular effects attributable to administration of vehicle at these volumes.

Results

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

Administration of AM 251 had no significant cardiovascular effects, although there were some behavioural actions, notably grooming, which influenced cardiovascular variables at the time they occurred, but which had generally passed before administration of WIN-55212-2 was started. Thus, resting haemodynamic variables in this group of animals ($n=8$) prior to administration of the first dose of WIN-55212-2 in the presence of vehicle or AM 251 were not significantly different (heart rate, 346 ± 15 and 336 ± 13 beats min^{-1}; mean arterial blood pressure, 102 ± 3 and 104 ± 4 mm Hg; renal vascular conductance, 86 ± 3 and 86 ± 4 (kHz mmHg^{-1})10^3; mesenteric vascular conductance, 105 ± 8 and 109 ± 10 (kHz mmHg^{-1})10^3; hindquarters vascular conductance, 35 ± 2 and 39 ± 5 (kHz mmHg^{-1})10^3), respectively). As noted previously (Gardiner *et al*., 2001), in the presence of vehicle, WIN-55212-2 had variable effects on heart rate, but caused a dose-dependent pressor response associated with renal and mesenteric vasoconstriction and hindquarters vasodilatation (Figure 1). All the significant cardiovascular effects of WIN-55212-2 were markedly inhibited by AM 251 (Figure 1).

Cardiovascular responses to HU 210 in the absence or presence of AM 251

In the first experiment, one group of animals was given HU 210 (10 μg kg^{-1}) on Day 1, vehicle on Day 2 and HU 210 (100 μg kg^{-1}) on Day 3. There were no cardiovascular effects of HU 210 (10 μg kg^{-1}) which differed from the vehicle (data not shown). The responses to HU 210 (100 μg kg^{-1}) were generally similar to those obtained when HU 210 (100 μg kg^{-1}) was given to naïve rats (see below), but there were some differences which may have been due to de-sensitization, following exposure to the lower dose of the drug on Day 1 (data not shown).

Administration of AM 251 had no significant cardiovascular actions and, hence, cardiovascular variables immediately prior to administration of HU 210 (100 μg kg^{-1}) in the presence ($n=7$) or absence ($n=6$) of AM 251 were similar, and not different to those in the animals ($n=7$) given the vehicle for HU 210 in the presence of AM 251 (heart rate, 320 ± 10, 331 ± 12 and 326 ± 11 beats min^{-1}; mean arterial blood pressure, 102 ± 2, 101 ± 3 and 107 ± 3 mm Hg; renal vascular conductance, 84 ± 6, 87 ± 10 and 103 ± 7 (kHz mmHg^{-1})10^3; mesenteric vascular conductance, 99 ± 6, 100 ± 14 and 95 ± 6 (kHz mmHg^{-1})10^3; hindquarters vascular conductance, 44 ± 4, 39 ± 3 and 44 ± 5 (kHz mmHg^{-1})10^3), respectively.

Following administration of vehicle for AM 251, HU 210 (100 μg kg^{-1}) caused bradycardia, a rise followed by a fall in blood pressure, short-lived renal vasoconstriction, longer-lasting mesenteric vasoconstriction and marked, prolonged hindquarters vasodilatation (Figure 2). Following administration of AM 251, there was substantial inhibition of all the cardiovascular responses to HU 210 (Figure 2). Thus, over the 5 h recording period, there were no differences between the integrated changes in heart rate, blood pressure, or renal and mesenteric vascular conductances in the animals given AM 251 followed by vehicle for HU 210 and those given AM 251 followed by HU 210 (Figure 2). Moreover, the residual increase in hindquarters vascular conductance in response to HU 210 in the presence of AM 251 was significantly ($P\leqslant0.001$) less than in the absence of AM 251 (Figure 2).

The cardiovascular effects of WIN-55212-2 (150 μg kg^{-1}), administered at the end of the 5 h recording period, were markedly inhibited in the animals treated with AM 251 (e.g. integrated (0–5 min) increases in hindquarters vascular conductances were $+172\pm75$, $+30\pm18$ and $+33\pm13$% min in animals given vehicle for AM 251 followed by HU 210, AM 251 followed by HU 210 and AM 251 followed by vehicle for HU 210 respectively).

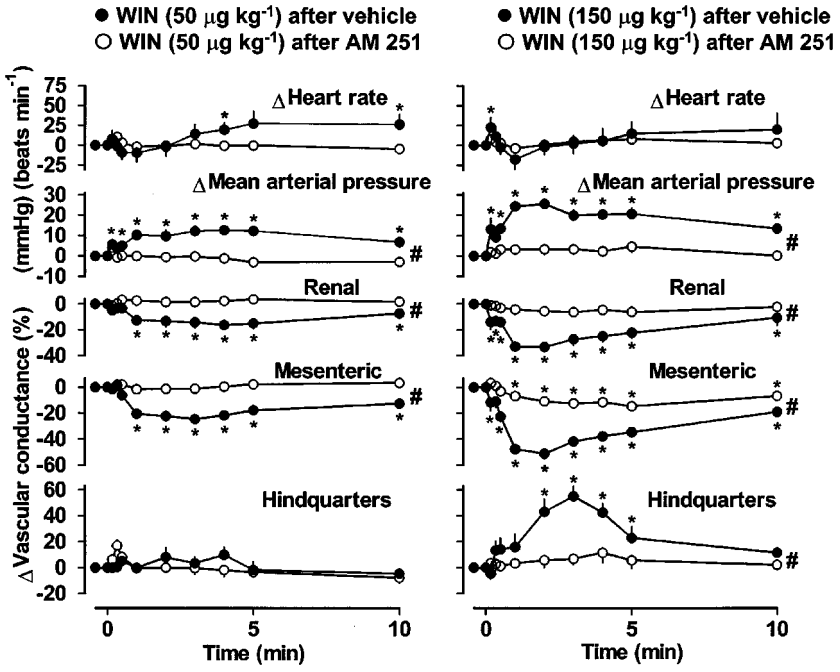

Figure 1 Cardiovascular responses to WIN-55212-2 (50 or 150 μg kg^{-1}) after vehicle or AM 251 (3 mg kg^{-1}) on different days in the same, conscious, Sprague-Dawley rats ($n=8$). Values are mean and vertical bars show s.e.mean; *$P\leqslant0.05$ *versus* baseline (Friedman's test); #$P\leqslant0.05$ for integrated responses after vehicle *versus* integrated responses after AM 251 (Wilcoxon test). WIN-55212-2 (50 μg kg^{-1}) after vehicle *versus* after AM 251: mean arterial pressure, $P\leqslant0.02$; renal vascular conductance, $P\leqslant0.02$; mesenteric vascular conductance, $P\leqslant0.02$. WIN-55212-2 (150 μg kg^{-1}) after vehicle *versus* after AM 251: mean arterial pressure, $P\leqslant0.02$; renal vascular conductance, $P\leqslant0.05$; mesenteric vascular conductance, $P\leqslant0.02$; hindquarters vascular conductance, $P\leqslant0.05$.

Cardiovascular responses to WIN-55212-2 in the absence or presence of ICI 118551

In pilot experiments ($n=2$) it was shown that the cardiovascular responses to administration of WIN-55212-2 (150 μg kg^{-1}) before and after a 90 min infusion of saline were consistent (changes in two animals at 3 min following WIN-55212-2 administration: blood pressure, before saline: +22 , +17 mmHg; after saline: +27, +22 mmHg; renal vascular conductance, before saline: −37, −28%; after saline: −43, −31%; mesenteric vascular conductance, before saline: −43, −39%; after saline: −42, −45%; hindquarters vascular conductance, before saline: +50, +63%; after saline: +61, +56%).

Resting cardiovascular variables in animals ($n=7$), prior to administration of WIN-55212-2 in the absence and presence of ICI 118551 were as follows:- heart rate, 337 ± 17 and 322 ± 12 beats min^{-1}; mean arterial blood pressure, 108 ± 7 and 110 ± 7 mm Hg; renal vascular conductance, 79 ± 8 and 76 ± 7 (kHz mmHg^{-1})10^3; mesenteric vascular conductance, 79 ± 6 and 74 ± 5 (kHz mmHg^{-1})10^3; hindquarters vascular conductance, 46 ± 4 and 38 ± 3 (kHz mmHg^{-1})10^3), respectively. Hindquarters vascular conductance was significantly ($P\leqslant0.01$) lower in the presence of ICI 118551 than in its absence, but the other cardiovascular variables were not different.

Responses to WIN-55212-2 in the absence and presence of ICI 118551 are shown in Figure 3. In the absence of ICI 118551, the cardiovascular responses to WIN-55212-2 (150 μg kg^{-1}) were as described above. In the presence of ICI 118551, the integrated pressor response to WIN-55212-2 and the associated falls in renal and mesenteric vascular conductances were not significantly affected, but the rise in hindquarters vascular conductance (+34±12% min) was significantly attenuated ($P\leqslant0.02$). In the presence of ICI 118551, the variable heart rate response to WIN-55212-2 was not significantly affected (Figure 3).

Cardiovascular responses to HU 210 in the presence of ICI 118551

Resting cardiovascular variables in rats ($n=8$) receiving ICI 118551, prior to the administration of HU 210 (100 μg kg^{-1}) were as follows: heart rate, 320 ± 17 beats min^{-1}, mean arterial blood pressure, 105 ± 5 mm Hg, renal vascular conductance, 99 ± 10 (kHz mmHg^{-1})10^3, mesenteric vascular conductance, 105 ± 12 (kHz mmHg^{-1})10^3, hindquarters vascular conductance, 43 ± 5 (kHz mmHg^{-1})10^3. Responses to HU 210 in the absence and presence of ICI 118551 are shown in Figure 3. In the presence of ICI 11551, the integrated (0–5 h) changes in heart rate, mean arterial blood and renal and mesenteric vascular conductances in response to HU 210 (100 μg kg^{-1}) were similar to those seen in the animals given HU 210 in the presence of the vehicle for AM 251 However, the increase in hindquarters vascular conductance in response to HU 210 in the presence of ICI 118551 was significantly ($P\leqslant0.001$) less than in its absence.

Discussion

The aims of this study were to compare the regional haemodynamic effects of the structurally different cannabi-

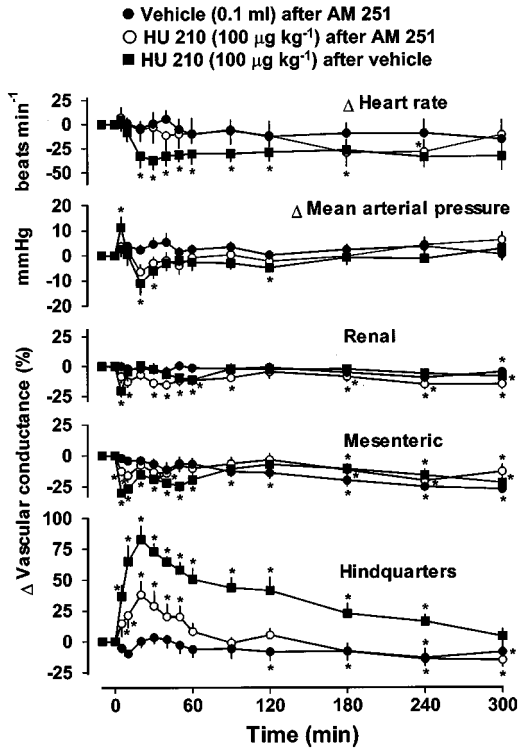

Figure 2 Cardiovascular responses to vehicle for HU 210 after AM 251 (3 mg kg^{-1}; $n=7$), or HU 210 (100 μg kg^{-1}) after AM 251 ($n=7$) or HU 210 (100 μg kg^{-1}) after vehicle for AM 251 ($n=6$). Values are mean and vertical bars show s.e.mean. *$P\leqslant 0.05$ *versus* baseline (Friedman's test). For clarity, between-group comparisons are not included on the Figure, but are mentioned in the text and detailed below. The only significant (Kruskal–Wallis test) differences were between the integrated changes in hindquarters vascular conductances: HU 210 after vehicle for AM 251 *versus* HU 210 vehicle after AM 251 ($p\leqslant 0.01$). HU 210 after vehicle for AM 251 *versus* HU 210 after AM 251 ($P\leqslant 0.001$). HU 210 vehicle after AM 251 *versus* HU 210 after AM 251 ($P\leqslant 0.01$).

noid agonists, HU 210 and WIN-55212-2, in conscious rats, and to examine the extent of involvement of cannabinoid receptors in the cardiovascular responses observed. In addition, we explored the possible contribution of β_2 adrenoceptors to cannabinoid-induced hindquarters vasodilatation.

The most notable findings are that: (i) HU 210 and WIN-55212-2 caused similar patterns of cardiovascular change (pressor, renal and mesenteric vasoconstrictor, hindquarters vasodilator), but with markedly different time courses; (ii) the cardiovascular effects of HU 210 and WIN-55212-2 were substantially antagonized by AM 251, although the latter had no significant effects on resting haemodynamics; (iii) the hindquarters vasodilator responses to WIN-55212-2 and HU 210, which appeared to involve CB_1-receptors, were susceptible to β_2-adrenoceptor antagonism.

Previously (Gardiner *et al*., 2001), we have drawn attention to the fact that, in conscious rats, the haemodynamic effects of WIN-55212-2 are quite different from those reported in anaesthetized rats (Lake *et al*., 1997), but resemble, in some regards, the effects reported in conscious rabbits (Niederhoffer & Szabo, 1999, 2000). Here we show, again, dose-dependent pressor, renal and mesenteric vasoconstrictor and hindquarters vasodilator effects of WIN-55212-2 and, in addition, now demonstrate marked inhibition of those effects by the CB_1-receptor antagonist, AM 251 (Gatley *et al*., 1996; 1997).

To our knowledge, this is the first study to describe antagonism of cannabinoid-mediated cardiovascular effects by AM 251 *in vivo*. Most studies involving CB_1-receptor antagonists *in vivo* have used SR 141716A, which is not commercially available. AM 251 and AM 281 are analogues of SR 141716A, which bind with high affinity to cannabinoid CB_1-receptors following *in vivo* administration (Gatley *et al*., 1996, 1997). We chose to give a dose of 3 mg kg^{-1} AM 251, since that was the dose of SR 141716A used by Lake *et al*. (1997) which blocked the cardiovascular effects of cannabinoid agonists *in vivo*, and because Izzo *et al*. (2000) showed equivalent antagonism by equimolar doses of SR 141716A and AM 281 *in vitro*. It is clear from our results that we had achieved effective cannabinoid receptor antagonism, but it is notable that AM 251 itself had no significant cardiovascular effects. This suggests that cannabinoid receptor-mediated events are unlikely to be involved in normal cardiovascular regulation. Interestingly, we have recently shown that AM 251, at the dose used here, has no effect on the cardiovascular actions of exogenous anandamide (Gardiner *et al*., 2002a), suggesting that the latter may not involve cannabinoid receptors.

We reported earlier that the pressor and vasoconstrictor effects of WIN-55212-2 were susceptible to ganglion blockade and were, therefore, likely to be due to sympathoexcitation (Gardiner *et al*., 2001). The present results indicate that these effects are CB_1-receptor-mediated, but do not allow us to localize the cannabinoid receptors involved although, on the basis of the work of others, we would expect this to be a centrally-mediated action (Niederhoffer & Szabo, 1999, 2000). Our earlier work showed that the hindquarters vasodilator effect of WIN-55212-2 was not susceptible to ganglion blockade (indeed, it was enhanced under those conditions), and we speculated about a possible involvement of β_2-adrenoceptors.

We have previously shown (Gardiner *et al*., 1992), and confirmed here, that administration of a β_2-adrenoceptor agonist to conscious rats causes selective vasodilatation in the hindquarters, and that the dose of ICI 118551 used presently ablates that response. Our present findings, which show that both AM 251 and ICI 118551, independently, antagonize the hindquarters vasodilator response to WIN-55212-2, are therefore entirely consistent with an involvement of β_2-adrenoceptors in the CB_1-receptor-mediated hindquarters vasodilator response. Although one theoretical possibility is that CB_1-receptors stimulate adrenal medullary adrenaline release, this is not consistent with the only published paper that we are aware of on the topic (Niederhoffer *et al*., 2001), which shows that, in pithed rabbits, cannabinoids inhibit adrenaline release.

Although the time course of the effect of HU 210 was far longer than that of WIN-55212-2, which is consistent with the higher affinity of binding of the former to CB_1-receptors (Pertwee, 1997), the patterns of response to the cannabinoids were remarkably similar, inasmuch as both had pressor and renal and mesenteric vasoconstrictor effects, accompanied by hindquarters vasodilatation, and all these effects were antagonized by AM 251. Furthermore, the hindquarters vasodilator effect of HU 210, like that of WIN-55212-2 was reduced substantially by ICI 118551.

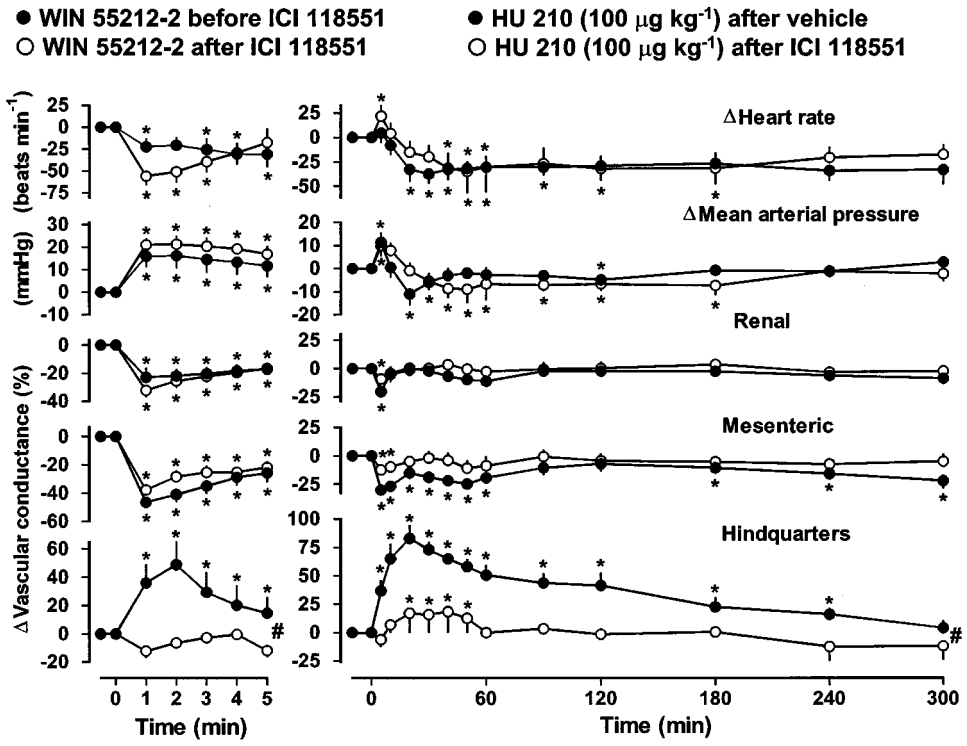

Figure 3 Effects of ICI 118551 (0.2 mg kg^{-1}, 0.1 mg kg^{-1} h^{-1}) on the cardiovascular responses to WIN-55212-2 (150 μg kg^{-1}) and HU 210 (100 μg kg^{-1}). Values are mean and vertical bars show s.e.mean. *$P \leqslant 0.05$ *versus* baseline (Friedman's test). Responses to WIN-55212-2 were obtained in one group of animals ($n=7$) before and 90 min following administration of ICI 118552. #$P \leqslant 0.02$ for integrated (0–5 min) response (Wilcoxon test). Responses to HU 210 in the presence of ICI 118551 ($n=8$) were compared to those obtained in the rats ($n=6$) given HU 210 following the vehicle for AM 251 (data from Figure 2). #$P \leqslant 0.001$ for integrated (0–300 min) response (Mann–Whitney test).

It is worth noting that, whilst an increase in locomotor activity would result in hindquarters vasodilatation, that cannot be the explanation for our results since, particularly following administration of HU 210, locomotor activity was substantially suppressed.

Our results with HU 210 differ in some respects from those in the literature, where prolonged hypotension and bradycardia following administration of HU 210 has been described (Vidrio *et al*., 1996; Lake *et al*., 1997). For example, in anaesthetized rats, HU 210 (100 μg kg^{-1}) has been reported to cause a fall in blood pressure of $\sim$50 mmHg which was sustained for the duration of the 90 min recording period (Vidrio *et al*., 1996). In the recent report of Wagner *et al*. (2001) it was suggested that the hypotension caused by HU 210 was entirely due to a fall in cardiac output. Furthermore, it was proposed that the underlying mechanism was a cannabinoid receptor-mediated prejunctional inhibition of cardiac sympathetic tone, since the fall in cardiac output was not evident in ganglion-blocked animals (Wagner *et al*., 2001). However, in the ganglion-blocked state, there was still a substantial fall in blood pressure in response to HU 210 (−57 mm Hg) which was not accounted for either by a fall in cardiac output, or by peripheral vasodilatation. Wagner *et al*. (2001) did not explain these findings, and we cannot see an obvious explanation for their discordant results. However, considering our results against the background of published findings it seems that responses to HU 210, like those of WIN-55212-2 (see Introduction), may be influenced substantially by anaesthesia. Thus, it is unfortunate that the unratified dogma is that cannabinoids are hypotensive and vasodilator, considering this view derives from studies in anaesthetised animals, and is being used as a basis for proposing that cannabinoids might be useful in treating hypertension (e.g., Porter & Felder, 2001).

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