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Evidence of a novel site mediating anandamide-induced negative inotropic and coronary vasodilatator responses in rat isolated hearts

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1 Cannabinoids are known to cause coronary vasodilatation and reduce left ventricular developed pressure (LVDP) in isolated hearts although the identity of the receptor(s) mediating these responses is unknown. Our objective was to pharmacologically characterize cannabinoid receptors mediating cardiac responses to the endocannabinoid, anandamide.

2 Dose-response curves for coronary perfusion pressure (CPP) and LVDP were constructed to anandamide, R-(+)-methanandamide, palmitoylethanolamide (PEA) and JWH015 in isolated Langendorff-perfused rat hearts. Anandamide dose-response curves were also constructed in the presence of antagonists selective for CB_1, CB_2 or VR_1 receptors.

3 Anandamide and methanadamide significantly reduced CPP and LVDP but the selective CB_2 receptor agonists, PEA and JWH015 had no significant effect, compared with equivalent vehicle doses.

4 Single bolus additions of the selective CB_1-receptor agonist, ACEA (5 nmol), decreased LVDP and CPP. When combined with JWH015 (5 nmol) these responses were not augmented.

5 Anandamide-mediated reductions in CPP were significantly blocked by the selective CB_1 receptor antagonists SR 141716A (1 μM) and AM251 (1 μM) and the selective CB_2 receptor antagonist SR 144528 (1 μM) but not by another selective CB_2 receptor antagonist AM630 (10 μM) nor the vanilloid VR_1 receptor antagonist capsazepine (10 μM).

6 SR 141716A, AM281 and SR 144528 significantly blocked negative inotropic responses to anandamide that were not significantly affected by AM251, AM630 and capsazepine.

7 One or more novel sites mediate negative inotropic and coronary vasodilatatory responses to anandamide. These sites can be distinguished from classical CB_1 and CB_2 receptors, as responses are sensitive to both SR 141716A and SR 144528.

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Abbreviations: Δ^9-THC, Δ^9-tetrahydrocannabinol; ACEA Arachidnyl-2′-choloroethylamide; ANOVA, Analysis of variance; CPP, Coronary perfusion pressure; PEA, Palmitoylethanolamide; LVDP, Left ventricular developed pressure

Introduction

Numerous reports of cardiac responses to plant-derived cannabinoids were published between 1971 and 1985 (Benmouyal *et al*., 1971; Huy *et al*., 1972; Manno & Manno, 1973; Nahas & Trouve, 1985). Since then, endogenous cannabinoids have been discovered (Devane *et al*., 1992; Mechoulam *et al*., 1995; Sugiura *et al*., 1995) and two distinct receptor subtypes identified and cloned, namely CB_1 (Matsuda *et al*., 1990) and CB_2 (Munro *et al*., 1993). Concurrent with these discoveries has been the development of agonists and antagonists selective for CB_1 and CB_2 receptors that have been extensively used to pharmacologically classify responses to endocannabinoids (Pertwee, 2000).

When studied *in vivo*, endocannabinoids in the cardiovascular system cause hypotension and bradycardia mediated *via* CB_1 receptors (Járai *et al*., 2000; Lake *et al*., 1997). *In vitro* experiments have reported that endocannabinoids cause vasodilatation in cerebral arteries (Ellis *et al*., 1995; Gebremedhin *et al*., 1999), mesenteric arteries (Randall *et al*., 1996; White & Hiley, 1997), juxtamedullary afferent arterioles (Deutsch *et al*., 1997), vas deferens (Lay *et al*., 2000) and coronary arteries (Pratt *et al*., 1998; Randall & Kendall, 1997; White *et al*., 2001). Most of the evidence supporting the hypothesis that endocannabinoid-induced vasodilatation is mediated by stimulation of CB_1 receptors is based on sensitivity to antagonism with SR 141716A (Gebremedhin *et al*., 1999; Harris *et al*., 1999; Járai *et al*., 2000; Lay *et al*., 2000; Wagner *et al*., 1999). However, SR 141716A-independent vasodilatation in response to endocannabinoids has also been reported (Járai *et al*., 1999; Pratt *et al*., 1998; White & Hiley, 1998a, b; White *et al*., 2001; Zygmunt *et al*., 1999).

Endocannabinoids reduce blood pressure and decrease heart rate *in vivo via* activation of peripheral CB_1 receptors (Járai *et al*., 2000; Lake *et al*., 1997; Varga *et al*., 1995). To date, the effects of endocannabinoids on cardiac function in isolated hearts have been limited to study of coronary

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vasodilatation (Fulton & Quilley, 1998; Randall & Kendall, 1997). The effects of plant-derived cannabinoids, such as Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabinol, are known to reduce left ventricular contractile force (Nahas & Trouve, 1985; Smiley *et al*., 1976).

CB_2 receptors have not been thought to mediate cardiovascular responses to endocannabinoids, as their expression is generally believed to be limited to the immune system (Munro *et al*., 1993). However, it has recently been reported that CB_2 receptors might be involved in cardiac preconditioning against ischaemia-reperfusion injury (Lagneux & Lamontagne, 2001).

In addition to CB_1 and CB_2 receptors, endocannabinoids have been reported to act as agonists of vanilloid VR1 receptors (Ross *et al*., 1999; Smart *et al*., 2000) mediating arterial vasodilatation in rats and guinea-pigs (Zygmunt *et al*., 1999). These findings were made on the basis of sensitivity of endo-cannabinoid responses to the VR1 receptor antagonist cap-sazepine. The potential role of VR1 receptors in mediating cardiac responses to endocannabinoids has not been directly studied.

The last recognized pathway by which responses to endocannabinoids can be mediated is through uptake and hydrolysis to form arachidonic acid (Craib *et al*., 2001; Deutsch & Chin, 1993; Di Marzo *et al*., 1994). This increase in arachidonic acid levels might lead to eicosanoid production that may have a role in mediating responses to anandamide (Ellis *et al*., 1995; Craib *et al*., 2001). However, it is questionable whether this pathway contributes significantly to observed responses to endocannabinoids (Jarrahian & Hillard, 1997).

Despite the discovery of CB_1 and CB_2 receptors and the development of selective agonists and antagonists, little attempt has been made to classify pharmacologically endocannabinoid effects directly on the heart. We have recently reported that anandamide causes vasorelaxation of isolated coronary arteries by a mechanism independent of CB_1, CB_2 or VR_1 receptors (White *et al*., 2001). Therefore, we have extended this previous work to identify the cannabinoid receptors that mediate inotropic and coronary vascular responses to anandamide in the rat isolated Langendorff-perfused heart.

Methods

Langendorff perfusion

All animals were housed and treated in accordance with the Animals (Scientific Procedures) Act 1986. Male Sprague-Dawley rats (300–400 g), that had been fed *ad libitum*, were killed with an overdose of sodium pentobarbitone (Rhône Mérieux) and heparinized (100 u kg^{-1} i.p, CP Pharmaceuticals, U.K.). Hearts were rapidly excised and placed in ice-cooled Krebs-Henseleit solution.

Hearts were mounted on a Langendorff perfusion apparatus by cannulating the aorta within 90 s of excision. Constant flow (10 ml min^{-1}) Langendorff perfusion was then commenced using a Cole-Parmer Masterflex peristaltic pump (model 7553-75). The rate of perfusion was constantly monitored with ultrasonic flow probes (Transonic H4X) placed on the inflow line. The perfusate, consisting of a modified Krebs-Henseleit solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 11 mM glucose, 100 mU l^{-1} insulin) was maintained at 37°C and continuously bubbled with a gas mixture of 95% O_2/5% CO_2. Hearts were immersed in a chamber containing perfusate at 37°C and electrically paced *via* bipolar platinum electrodes at a frequency of 5 Hz (Palmer Bioscience Stimulator 100).

Left ventricular developed pressure was measured by means of a pressurized balloon (Harvard Apparatus) inserted into the left ventricle, connected to a pressure transducer (Ohmeda, Singapore, model P23XL-1) and inflated to a level such that end diastolic pressure was set to a value between 5–10 mmHg. All parameters were continuously recorded using a PowerLab 800 (ADInstruments) and stored using a Macintosh PowerPC.

Experimental protocol and drugs used

Graded doses of anandamide, R-(+)-methanandamide, JWH015 or palmitoylethanolamide (Tocris Cookson Ltd, Bristol, U.K.) were added in boluses of 1 ml in a range from 0.03 to 3 μmol. Doses were loaded into the perfusate line before the perisaltic pump in order to eliminate the pressure artifact due to bolus addition. All agonists were prepared in a vehicle consisting of 1 : 4 soya oil : water mixture emulsified with poloxamer F188 (gift from Dr Washington, Institute of Pharmaceutical Sciences, University of Nottingham, UK) and subsequently serially diluted in Krebs-Henseleit solution. Varying volumes of vehicle, equivalent to those used in the serial dilutions of the agonists, made up to a 1 ml bolus with Krebs-Henseleit solution, were also tested.

Single doses of drug vehicle, arachidonyl-2′-choloroethylamide (ACEA, 5 nmol, Tocris Cookson Ltd) or a mixture of ACEA (5 nmol) + JWH015 (5 nmol) were added in boluses of 10 μl proximal to the heart. ACEA and JWH017 were dissolved in 100% ethanol. The order of bolus addition was predetermined according to a randomized block design. Responses were measured 5 min after bolus addition.

Stock solutions (1 mM) of AM251 (Tocris Cookson Ltd), AM281 (Tocris Cookson Ltd), AM630 (Tocris Cookson Ltd), SR 141716A (gift from Sanofi Synthelabo, France), SR 144528 (gift from Sanofi Synthelabo, France) and capsazepine (Sigma, Poole, U.K.) were initially prepared in DMSO then subsequently diluted in the Krebs–Henseleit perfusate (final concentrations of AM251, AM281, SR 141716A and SR 144528 were 1 μM, AM630 and capsazepine were 10 μM). The final concentration of DMSO in the perfusate was 0.2% (v v^{-1}). Hearts were allowed to equilibrate for 30 min before construction of an agonist dose-response curve.

Hearts were excluded from the study if LVDP <60 mmHg and/or CPP >100 mmHg after 10 min of perfusion. No hearts were excluded from the current study.

Statistics

Data are expressed as means ± s.e.m. Statistical differences between baseline values were determined by analysis of variance (ANOVA). Differences between dose-response curves and single bolus additions were determined by ANOVA with repeated measures followed by Bonferroni's *post hoc* test. Statistical significance was taken to be $P<0.05$. All statistical calculations were carried out with StatView 4.5 for the Macintosh (Abacus Concepts, Inc., Berkeley, California, U.S.A.).

Results

Effects of cannabinoid receptor agonists on left ventricular developed pressure

Baseline left ventricular developed pressure (LVDP) did not vary significantly among the different treatment groups (Table 1). Addition of vehicle had no effect on LVDP at any of the doses used. Responses to the selective CB_2 receptor agonists, JWH015 and palmitoylethanolamide, did not significantly differ from equivalent vehicle treatments (Figure 1).

Anandamide caused a significant dose-dependent decrease in LVDP. The response at the highest dose of anandamide (3 μmol) did not appear to be maximal (Figure 1A), reducing LVDP by 26 ± 5 mmHg ($n=6$). R-(+)-methanandamide also significantly reduced LVDP in a dose-dependent manner. The highest dose of R-(+)-methanandamide (3 μmol) similarly did not appear to result in a maximal response (Figure 1A), reducing LVDP by 32 ± 4 mmHg ($n=6$).

Effects of cannabinoid receptor agonists on coronary perfusion pressure

Baseline coronary perfusion pressure (CPP) did not vary among the different treatment groups (Table 1). Graded dilutions of vehicle, equivalent to those used in serial dilutions of the agonists, delivered as 1 ml boluses caused a small increase in CPP (Figure 1B). The effects of the CB_2 receptor-selective agonists, JWH015 and palmitoylethanolamide, on CPP were not statistically different from the equivalent vehicle treatments.

Anandamide caused a dose-dependent decrease in CPP (Figure 1B) that was significantly different from the equivalent vehicle treatments. The highest dose of anandamide used (3 μmol) did not apparently give a maximal response, but reduced CPP by 11 ± 4 mmHg ($n=6$). R-(+)-methanandamide similarly caused a significant dose-dependent reduction in CPP compared to the equivalent vehicle treatments (Figure 1B). At the highest dose used (3 μmol), R-(+)-methanandamide caused a 6 ± 1 mmHg ($n=6$) fall in CPP.

Effects of antagonists on anandamide-induced decreases in left ventricular developed pressure

None of the antagonists used affected baseline LVDP prior to anandamide treatment (Table 1). The presence of 0.2% (v v^{-1}) DMSO in the perfusate had no significant effect on the dose-response curve to anandamide. Dose-response curves constructed to anandamide in the presence of AM251 (1 μM) were not significantly different from those obtained to anandamide alone (Figure 2A). Anandamide-induced inhibition of LVDP was abolished by the presence of the CB_1 selective antagonist, SR 141716A (1 μM) and significantly inhibited in the presence of 1 μM AM281 (Figure 2A). The CB_2 receptor-selective antagonist, AM630 (1 μM),

Table 1 Baseline values for coronary perfusion pressure (CPP) and left ventricular developed pressure (LVDP)

	n	*CPP* (mmHg)	*LVDP* (mmHg)
Vehicle	8	68±8	84±8
Anandamide	6	65±9	99±8
R-(+)-methanandamide	6	57±6	92±13
PEA	6	70±13	90±6
JWH015	6	67±4	92±3
Anandamide + AM251	6	54±4	102±7
Anandamide + AM281	6	64±7	89±5
Anandamide + AM630	4	71±8	97±8
Anandamide + SR141716A	6	65±7	83±12
Anandamide + SR144528	5	65±7	92±7
Anandamide + capsezpine	5	67±4	96±11

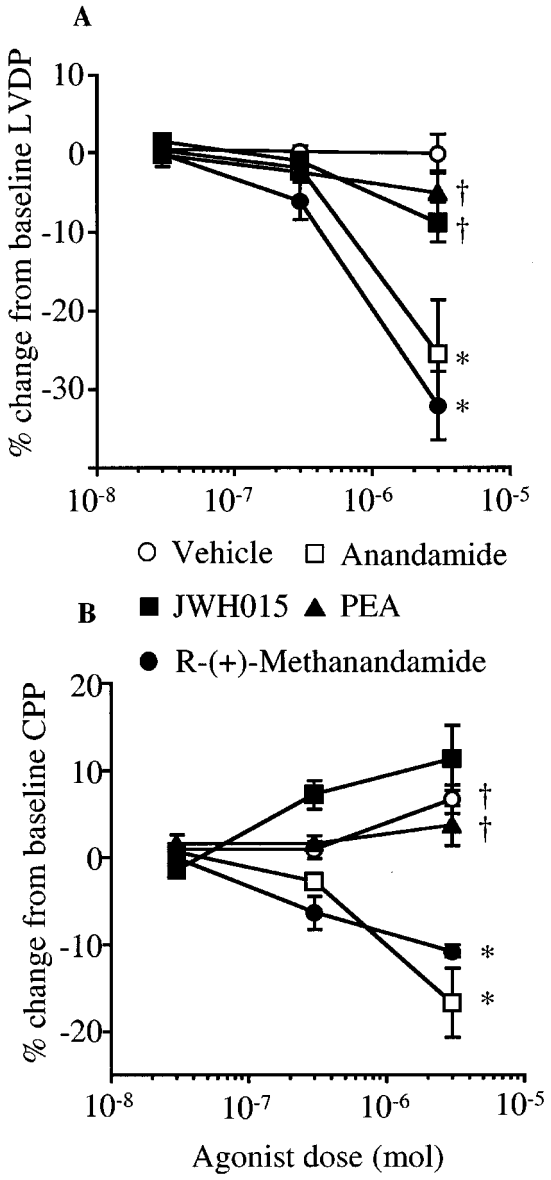

Figure 1 Dose-response curves for the effects of anandamide ($n=6$), R-(+)-methanandamide ($n=6$), palmitoylethanloamine (PEA, $n=6$) and JWH015 ($n=6$) on (A) left ventricular developed pressure (LVDP) and (B) coronary perfusion pressure (CPP). The effect of equivalent amounts of vehicle to that given with the various agonists are also shown ($n=7$). * Indicates significant differences ($P<0.05$) between agonist dose-response curves and the effects of equivalent vehicle doses (ANOVA with repeated measures followed by Bonferroni's *post hoc* test). †Indicates significant differences ($P<0.05$) between agonist dose response curves and those of anandamide (ANOVA with repeated measures followed by Bonferroni's *post hoc* test).

had no significant effect on LVDP responses to anandamide but the CB_2 selective anatagonist, SR 144528 (1 μM), abolished anandamide responses (Figure 2B). The vanilloid receptor antagonist, capsazepine (10 μM) had no effect on anandamide-induced negative inotropy (Figure 2C).

Effects of antagonists on anandamide-induced decreases in coronary perfusion pressure

Baseline CPP was not affected by any of the antagonists used (Table 1). The presence of 0.2% (v v^{-1}) DMSO in the perfusate had no significant effect on the dose-response curve to anandamide. AM281(1 μM) had no significant effect on anandamide-mediated reductions of CPP (Figure 3A). However, AM251 (1 μM), caused a significant attenuation of the anandamide dose-response curve (Figure 3A). Responses to anandamide were abolished by the presence of the CB_1 antagonist, SR 141716A (1 μM, Figure 3A). Anandamide dose-responses were unaffected by the presence of 10 μM of the CB_2 receptor-selective antagonist AM630 (Figure 3B). The CB_2 selective antagonist SR 144528 (1 μM) abolished the anandamide-induced negative inotropic responses (Figure 3B). Anandamide dose-responses were unaffected by 10 μM of the vanilloid receptor antagonist capsazepine, did not significantly affect anandamide-induced reductions in CPP (Figure 3C).

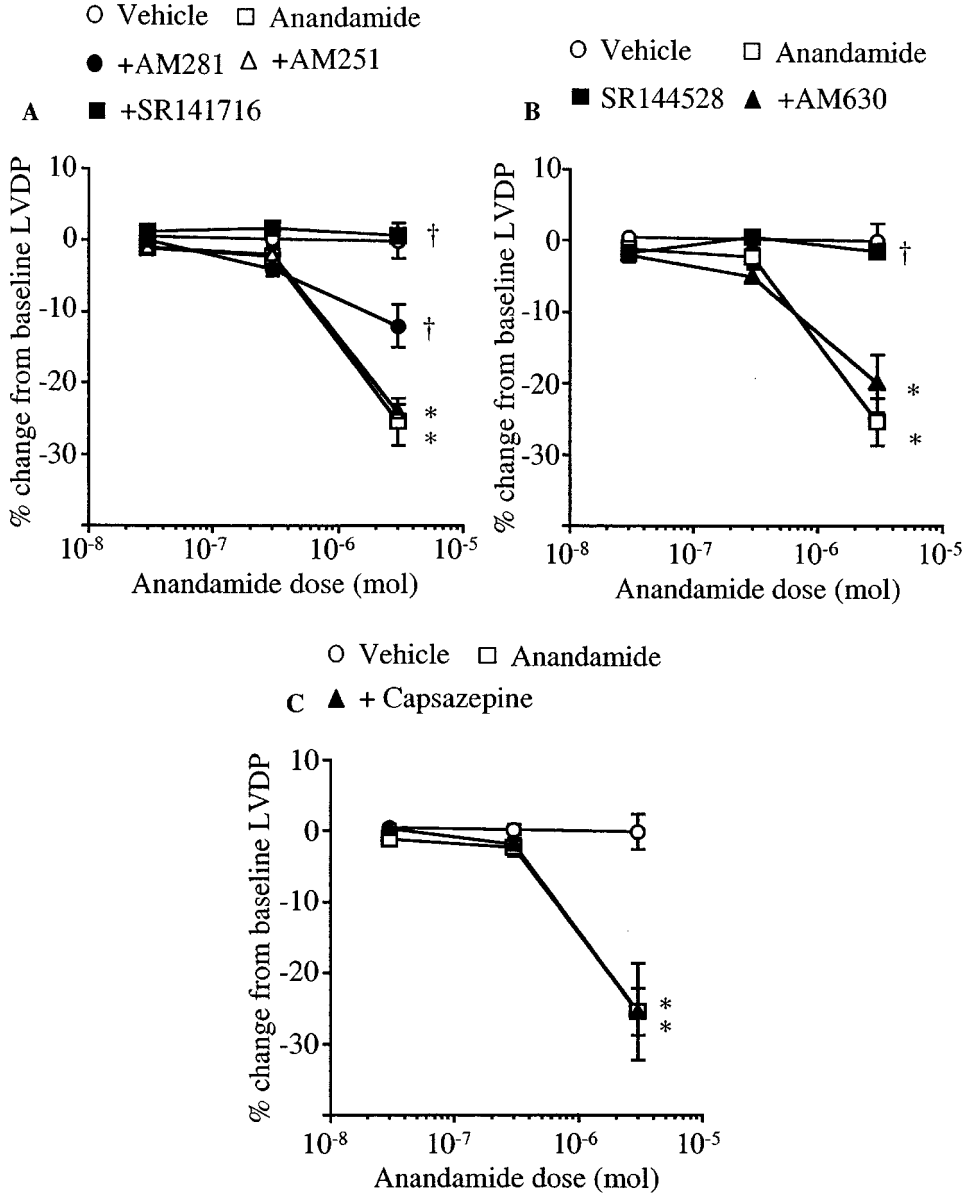

Figure 2 Dose-response curves for the effect of anandamide ($n=6$) on left ventricular developed pressure (LVDP) in the presence of (A) 1 μM AM281 ($n=6$), 1 μM AM251 ($n=6$) and 1 μM SR 141716A ($n=6$) or (B) 1 μM SR 144528 ($n=5$) and 10 μM AM630 ($n=4$) or (C) 10 μM capsazepine ($n=5$). The effect of equivalent amounts of vehicle to that given with various doses of anandamide are also shown ($n=7$). *Indicates significant difference ($P<0.05$) between dose response curve and the effects of equivalent vehicle doses (ANOVA with repeated measures followed by Bonferroni's *post hoc* test). †Indicates significant differences ($P<0.05$) between agonist dose response curves and those of anandamide (ANOVA with repeated measures followed by Bonferroni's *post hoc* test).

Effects of single bolus administrations of selective CB_1 and CB_2 receptor agonists

The addition of 100% ethanol (100 μl) caused a large transient decrease in both LVDP and CPP that rapidly (within 1 min) recovered to levels not significantly different from baseline. Addition of an ACEA bolus (5 nmol) significantly reduced LVDP and CPP (Figure 4). Responses to a 10 μl mixture of ACEA (5 nmol) and JWH015 (5 nmol) were not significantly different from those obtained with ACEA alone (Figure 4).

Discussion

The main finding of this study is that anandamide-induced coronary vasodilatation and negative inotropy in rat isolated hearts cannot be explained by any of the known mechanisms previously reported to mediate cannabinoid responses. Therefore, it would appear that one or more novel sites, distinct from either CB1 or CB2, mediate coronary vasodilatatory and negative inotropic responses to anandamide. These results may indicate the existence of a novel cannabinoid receptor subtype

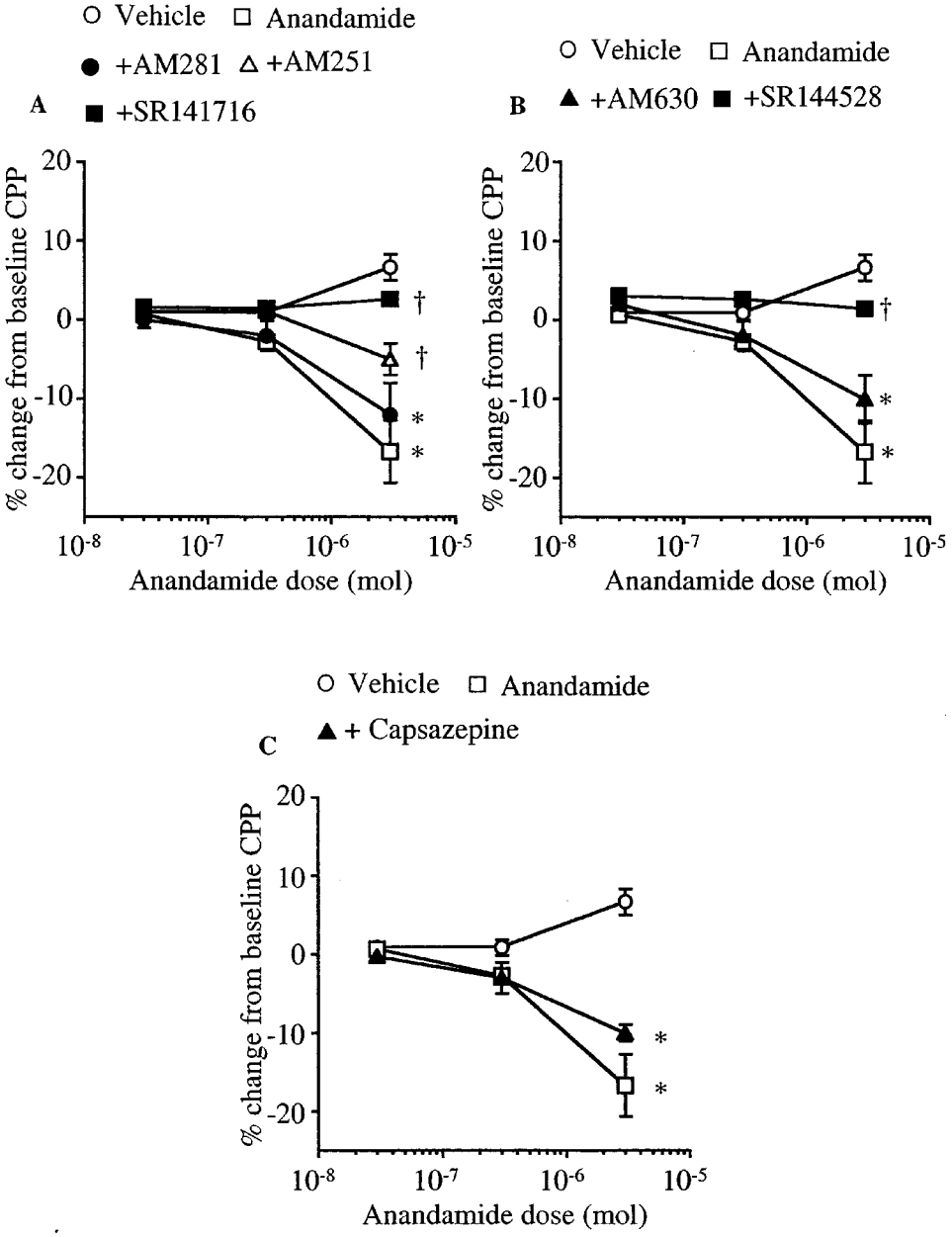

Figure 3 Dose-response curves for the effect of anandamide ($n=6$) on coronary perfusion pressure (CPP) in the presence of (A) 1 μM AM281 ($n=6$), 1 μM AM251 ($n=6$) and 1 μM SR 141716A ($n=6$) or (B) 1 μM SR 144528 ($n=5$) and 10 μM AM630 ($n=4$) or (C) 10 μM capsazepine ($n=5$). The effect of equivalent amounts of vehicle to that given with various doses of anandamide are also shown ($n=7$). *Indicates significant difference ($P<0.05$) between dose response curve and the effects of equivalent vehicle doses (ANOVA with repeated measures followed by Bonferroni's *post hoc* test). †Indicates significant differences ($P<0.05$) between agonist dose response curves and those of anandamide (ANOVA with repeated measures followed by Bonferroni's *post hoc* test).

mediating cardiac responses to anandamide in rat isolated hearts.

In agreement with previous studies (Fulton & Quilley, 1998; Randall & Kendall, 1997) we have shown that anandamide-induced coronary vasodilatation is abolished in the presence of 1 μM SR 141716A, a selective CB_1 receptor antagonist (Rinaldi-Carmona *et al*., 1996). However, unlike previous studies, we have found that anandamide-induced coronary vasodilatation is also abolished by the presence of 1 μM SR 144528, a selective CB_2 receptor antagonist (Rinaldi-Carmona *et al*., 1998). White & Hiley (1998a, b) have recently published evidence that SR 141716A, at concentrations of 10 μM, can cause responses *via* effectors other than the CB_1 receptor. In our study, we have used SR 141716A at a concentration of 1 μM that should be selective for CB_1 receptors. We are not aware of any reports that 1 μM SR 144528 has effects other than at the CB_2 receptor. Sensitivity of a response to SR 141716A and SR 144528 should be mutually exclusive if responses were mediated solely by CB_1 or CB_2 receptors. Our data clearly shows that responses to anandamide were sensitive to both SR 141716A and SR 144528. As this antagonism was observed with concentrations (1 μM) of SR 141716A and SR 144528 previously shown to be specific for cannabinoid receptors (Rinaldi-Carmona *et al*., 1996; 1998), it is likely that lack of selectivity is due to the involvement of an anandamide receptor(s), distinct from either the CB_1 or CB_2 subtypes, in mediating negative inotropy and coronary vasodilatation.

The inability of the selective CB_2 receptor antagonist, AM630 (Hosohata *et al*., 1997; Ross *et al*., 1999) to block anadamide responses and the lack of response to the selective CB_2 receptor agonists, JWH015 (Chin *et al*., 1999; Showalter *et al*., 1996) and palmitoylethanolamide (Calignano *et al*., 1998; Facci *et al*., 1995), provide further evidence that CB_2 receptors do not mediate coronary vasodilatation and negative inotropic responses to anandamide.

In addition to SR 141716A, we have tested the sensitivity of cardiac responses to anandamide with two other antagonists reported to be selective for CB_1 receptors, namely AM251 and AM281 (Gatley *et al*., 1998). Both AM251 and AM281 were used at concentrations (1 μM) sufficient to cause a 100 fold shift of a CB_1-receptor mediated dose-response curve and should have abolished responses to anandamide in the dose range we used. However, anandamide mediated coronary vasodilatation and negative inotropy was still observed in the presence of AM251 or AM281 indicating that these responses are not mediated by CB_1 receptors. Furthermore, anandamide-induced coronary vasodilatation was more sensitive to AM251 and anandamide-induced negative inotropy was more sensitive to AM281. This contrast in the effects of AM251 and AM281 suggests that it is possible there might be minor differences in the cannabinoid receptor populations mediating coronary vasodilatation and negative inotropic responses to anandamide.

As endocannabinoids can act as agonists of vanilloid VR1 receptors (Ross *et al*., 1999; Smart *et al*., 2000; Zygmunt *et al*., 1999), we tested if annadamide responses in the heart were sensitive to the selective VR1 receptor antagonist capsazepine (Bevan *et al*., 1992). Based on the published affinity of capsazepine for VR1 receptors (K_d of 220 nM, (Bevan *et al*., 1992)), 10 μM capsazepine should cause a 46 fold shift of a VR1-mediated dose-response curve. In this study, coronary vasodilatatory and negative inotropic responses to anandamide were unaffected by capsazepine. Therefore, responses to anandamide in the heart are not mediated by VR1 receptors.

It is known that anandamide can be taken up into a number of different cell types *via* a selective, saturable transport process (Bisogno *et al*., 1997; Di Marzo *et al*., 1994; Hillard *et al*., 1997) that is expressed in the cardiovascular system (Calignano *et al*., 1997). Intracellularly, anandamide is hydrolyzed to form ethanolamine and arachidonic acid (Deutsch & Chin, 1993; Di Marzo *et al*., 1994). Subsequent production of eicosanoids from arachidonic acid may mediate responses to anandamide (Ellis *et al*., 1995). Because the anandamide transport inhibitor, AM404, has been shown to activate VR1 receptors (Zygmunt *et al*., 2000) we chose to use R-(+)-methanandamide, the stable analogue of anandamide, to investigate whether hydrolysis to form arachidonic acid was involved mediating cardiac responses to anandamide. R-(+)-methanandamide elicited the same responses as anandamide, therefore the cardiac actions of anandamide do not appear to be mediated by its catabolism to arachidonic acid.

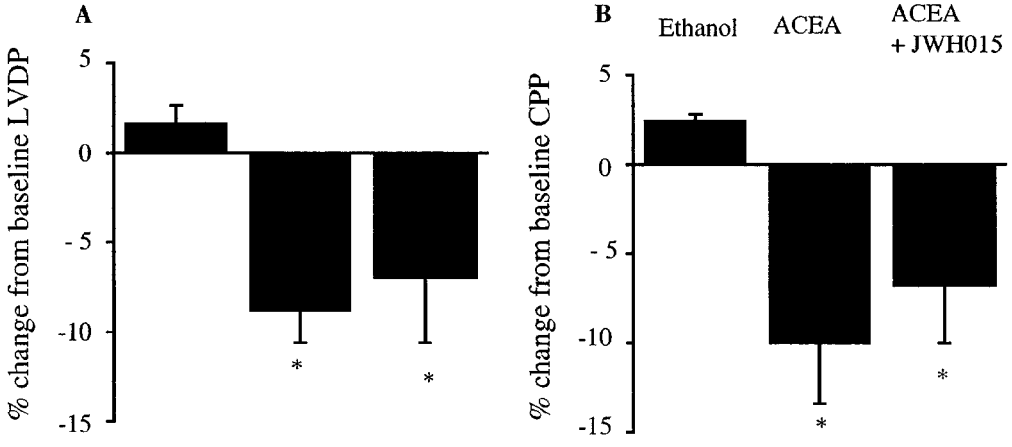

Figure 4 Effect of single doses of 5 nmol ACEA ($n=6$) or co-administration of 5 nmol ACEA with 5 nmol JWH015 ($n=6$) on (A) left ventricular developed pressure (LVDP) and (B) coronary perfusion pressure (CPP). *Indicates significant difference ($P<0.05$) between ethanol vehicle and ACEA or ACEA + JWH015 addition (ANOVA followed by Bonferroni's *post hoc* test).

We tested the possibility that anandamide responses might be dependent upon a synergistic activation of CB_1 and CB_2 receptors by comparing the effects of ACEA, a selective CB_1 receptor agonist (Hillard *et al*., 1999) with a combination of agonists selective for CB_1 (ACEA) and CB_2 (JWH015) receptors. Responses associated with the combination CB_1 and CB_2 receptor agonists did not lead to an augmentation of response over those seen with individual agonists alone. Therefore, the responses to the non-selective agonists, anandamide and methanandamide, are not due to synergistic activation of both CB_1 and CB_2 receptors.

The current data is in close agreement with our previous findings that anandamide relaxes isolated coronary arteries by a mechanism distinct from degradation into active metabolites or activation of CB_1, CB_2 or VR_1 receptors (White *et al*., 2001). The only minor difference between the two studies is that AM251 partially blocked anandamide-induced coronary vasodilatation in isolated hearts whereas it had no effect on anandamide-induced vasorelaxation in isolated coronary arteries (White *et al*., 2001).

In summary, anandamide and its stable analogue, R-(+)-methanandamide, induced negative inotropy and coronary vasodilatation. It was apparent that cardiac responses to anandamide were not mediated by any previously reported pathway. Therefore, we propose that anandamide causes coronary vasodilatation and negative inotropic responses by acting *via* one or more novel sites in rat isolated hearts. This novel anandamide site of action can be distinguished from classical CB_1 and CB_2 receptor subtypes on the basis of sensitivity to both SR 141716A and SR 144528 and might involve a new subtype of cannabinoid receptors.

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