RESEARCH PAPER

Acute administration of cannabidiol *in vivo* suppresses ischaemia-induced cardiac arrhythmias and reduces infarct size when given at reperfusion

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Background and purpose: Cannabidiol (CBD) is a phytocannabinoid, with anti-apoptotic, anti-inflammatory and antioxidant effects and has recently been shown to exert a tissue sparing effect during chronic myocardial ischaemia and reperfusion (I/R). However, it is not known whether CBD is cardioprotective in the acute phase of I/R injury and the present studies tested this hypothesis.

Experimental approach: Male Sprague-Dawley rats received either vehicle or CBD (10 or 50 μg·kg^{-1} i.v.) 10 min before 30 min coronary artery occlusion or CBD (50 μg·kg^{-1} i.v.) 10 min before reperfusion (2 h). The appearance of ventricular arrhythmias during the ischaemic and immediate post-reperfusion periods were recorded and the hearts excised for infarct size determination and assessment of mast cell degranulation. Arterial blood was withdrawn at the end of the reperfusion period to assess platelet aggregation in response to collagen.

Key results: CBD reduced both the total number of ischaemia-induced arrhythmias and infarct size when administered prior to ischaemia, an effect that was dose-dependent. Infarct size was also reduced when CBD was given prior to reperfusion. CBD (50 μg·kg^{-1} i.v.) given prior to ischaemia, but not at reperfusion, attenuated collagen-induced platelet aggregation compared with control, but had no effect on ischaemia-induced mast cell degranulation.

Conclusions and implications: This study demonstrates that CBD is cardioprotective in the acute phase of I/R by both reducing ventricular arrhythmias and attenuating infarct size. The anti-arrhythmic effect, but not the tissue sparing effect, may be mediated through an inhibitory effect on platelet activation.

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Introduction

Cannabinoids are a group of pharmacologically active agents, which consist of phytocannabinoids (plant-derived),

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endocannabinoids (endogenous) and synthetic cannabinoids. In relation to the phytocannabinoids, the parent plant *Cannabis sativa* consists of over 70 active compounds, the two most abundant being the psychoactive (-)-Δ^9-tetrahydrocannabinol (Δ^9-THC) and the non-psychoactive (-)-cannabidiol (CBD). In contrast to Δ^9-THC, CBD appears to act as an atypical cannabinoid at receptors typically activated by cannabinoids (reviewed by Pertwee, 2008). At low concentrations CBD has been shown to act as an inverse agonist at cannabinoid receptor 1 (CB$_1$), cannabinoid receptor 2 (CB$_2$)

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and possibly non CB_1/CB_2 receptors (Thomas et al., 2007), as an agonist at the transient receptor potential vanilloid type 1 (TRPV1; Bisogno et al., 2001) and 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$; Russo et al., 2005) receptors, and as an antagonist at the orphan receptor, G-protein-coupled receptor 55 (GPR55; Ryberg et al., 2007).

Although the precise pharmacological effects of CBD have yet to be fully elucidated, recent studies have demonstrated that it mediates a plethora of actions, including anti-inflammatory, antioxidant and anti-necrotic effects (reviewed by Mechoulam et al., 2007), all of which could confer tissue protective properties. For example CBD exerts an immuno-suppressive effect by decreasing tumour necrosis factor-α through enhanced endogenous adenosine signalling (Malfait et al., 2000) and prevents hydrogen peroxide (H_2O_2)-induced oxidative damage (Hampson et al., 1998). Moreover, CBD has been shown to inhibit mast cell uptake of anandamide (Rakhshan et al., 2000), which could explain observations that preservation of endocannabinoid levels ameliorates immunological-induced activation of mast cells (Vannacci et al., 2004), and suggests an additional anti-inflammatory role for CBD.

All of these anti-inflammatory actions of CBD would be predictive of a protective role in pathological events involving inflammation, such as ischaemia/reperfusion (I/R) injury. Indeed, a protective role for CBD, through a 5-HT$_{1A}$ receptor-dependent mechanism, in the setting of cerebral I/R injury has recently been demonstrated (Mishima et al., 2005). More recently, Durst et al. (2007) demonstrated that chronic administration of CBD significantly reduced myocardial infarct size measured several days following I/R and that this effect correlated with a pronounced anti-inflammatory effect, as evidenced by a reduced infiltration of inflammatory cells into the myocardium and serum levels of interleukin-6. Interestingly, this protection was not replicated in an ex vivo model of myocardial I/R, leading to the conclusion that the tissue sparing effects were not due to a direct action on the myocardium, but rather to prevention of a systemic inflammatory response. What is not known, however, is whether CBD exerts actions that influence events that occur in the early stages of myocardial ischaemia (such as the development of serious ventricular arrhythmias) and reperfusion (such as immediate tissue injury as opposed to delayed tissue injury).

Reports of the ability of CBD to interfere with some of the processes that play a central role in the early pathological events during I/R, such as platelet activation (Formukong et al., 1989) and ion channel opening (Mamas and Terrar, 1998) led us to predict that CBD may have wider cardioprotective potential than simply preventing the inflammatory response. The primary aim of this study was therefore to determine the effects of a single acute dose of CBD, both immediately prior to ischaemia onset and at the time of reperfusion, on cardiac arrhythmias and infarct size in a rat model of I/R. Because platelet activation (Flores et al., 1994) and mast cell degranulation (Walsh et al., 2009a,b) are two major contributors to arrhythmogenesis, and there have been reports of CBD affecting both these processes, the second aim was to explore whether or not any cardioprotective effects were accompanied by effects on platelet function and I/R-induced mast cell degranulation.

Methods

Coronary occlusion studies

Male Sprague-Dawley rats (300–400 g), were bred and housed in the University of Aberdeen Medical Research Facility. Animals were maintained at a temperature of $21 \pm 2°C$, with a 12 h light/dark cycle and with free access to food and tap water. Animals were obtained on a daily basis and allowed to acclimatize before commencing the study. All studies were performed under an appropriate Project License authorized under the UK Animals (Scientific Procedures) Act 1986.

Surgery. Animals were anaesthetized with pentobarbitone sodium salt (60 mg·kg^{-1} i.p; Sigma Aldrich, Poole, Dorset, UK) and the trachea cannulated to allow artificial respiration when required. The left carotid artery and the right jugular vein were cannulated with Portex polythene tubing (0.58 mm ID × 0.96 mm OD; Smiths Medical International Ltd., Hyde, Kent, UK). Arterial blood pressure was recorded via the left carotid artery using a pressure transducer (MLT844 Physiological Pressure Transducer; AD Instruments, Chalgrove, Oxfordshire, UK). A steel thermistor probe (Fisher Scientific Ltd., Loughborough, Leicestershire, UK) was inserted into the rectum to measure core temperature, which was maintained at 37–38°C with the aid of a Vetcare heated pad (Harvard Apparatus Ltd., Edenbridge, Kent, UK). The animal was then prepared for *in vivo* occlusion of the left anterior descending coronary artery (Clark et al., 1980) through a left thoracotomy, with rats ventilated on room air (54 strokes·min^{-1}; tidal volume, 1.5 mL per 100 g to maintain PCO_2 at 18–24 mmHg, PO_2 at 100–130 mmHg, and pH at 7.4; Harvard small animal respiration pump; Harvard Apparatus Ltd.). Anaesthesia was maintained throughout by administration of pentobarbitone sodium salt (3–4 mg·kg^{-1}) via the venous cannula every 30 min or as required. After placement of the ligature rats were allowed to stabilize for 15 min before drug/vehicle administration and subsequent coronary occlusion. The coronary artery was occluded (CAO) by tightening the ligature to induce regional ischaemia for 30 min, after which the ligature was loosened and the myocardium reperfused for 2 h. A standard limb lead I electrocardiogram (ECG) and mean arterial blood pressure (MABP) were monitored continuously throughout the experimental period using a Power Laboratory (AD Instruments) data acquisition system via a Bridge Amplifier (AD Instruments) and Animal Bio Amplifier (AD Instruments), respectively, and data subsequently analysed using Chart Software (AD Instruments). Any animals that had a starting MABP of <70 mmHg or developed spontaneous arrhythmias prior to CAO were excluded from the study.

Ex vivo *platelet aggregation studies.* Following completion of the I/R protocol, blood was withdrawn via the arterial cannula into a tube containing heparin (final blood concentration of 20 U·mL^{-1}). Platelet aggregation in response to collagen was then determined using whole blood impedance aggregometry (Chrono-log Aggregometer, Chrono-log Corporation, Havertown, PA, USA); 0.5 mL of whole blood was placed in a cuvette with 0.5 mL of saline (0.9% NaCl) at 37°C and stirred with a magnetic stir bar. Platelet aggregation (expressed in Ω)

in response to 5 µg·mL⁻¹ collagen was measured over a period of 10 min and data calculated using Aggrolink® software (Chrono-log Corporation).

Histological measurement of infarct size. Following blood withdrawal the rats were killed by an i.v. overdose of sodium pentobarbitone. The heart was then removed, the aorta cannulated and then gently perfused with saline (2 mL) to flush out residual blood. The ligature was then retied and Evans blue dye (2 mL; 0.5% w/v) perfused through the heart to delineate area at risk. Hearts were then removed and stored at –20°C prior to determination of infarct size. Frozen hearts were sliced into 2–3 mm slices from the apex to the base and allowed to defrost at room temperature. Myocardial tissue slices were then incubated in 1% triphenyltetrazonium chloride (Sigma Aldrich) in phosphate buffered saline for 15 min at 37°C to determine infarct size. Sections were then fixed in 10% buffered formal saline overnight and photographed using a SANYO VPC-E6U digital camera (SANYO Electric Co., Ltd., Osaka, Japan). Left ventricular area, area at risk, and infarct size were determined using computerized planimetry [ImageJ software, National Institute of Health (NIH), Rockville Pike, Bethesda, MD, USA]. Area at risk was expressed as a percentage of total left ventricular area, and infarct size was expressed as a percentage of area at risk.

Histological assessment of cardiac mast cell degranulation. Following infarct size measurement, myocardial tissue slices were embedded in paraffin wax (Thermo Scientific, Runcorn, Cheshire, UK) and 3 µm sections cut. Sections were dehydrated through a series of histosolve (Thermo Scientific) and graded alcohols and incubated in 0.1% w/v toluidine blue (Fisher Scientific Ltd.) at 37°C. After being stained, sections were mounted with a xylene substitute mountant (Thermo Scientific) and covered with a cover slip. Analysis of the tissue was carried out with the use of a Leica DMLB light microscope (Leica Microsystems, Milton Keynes, Bucks, UK) at a magnification of ×400. Mast cells were counted manually and the count encompassed the entire area of the tissue. Mast cell degranulation was determined as a loss of mast cell membrane integrity with extrusion of intracellular granules to the extracellular space or mast cells completely lacking in intracellular granules as described previously (Messina *et al.*, 2000).

Experimental protocols. Four experimental groups were used to investigate the effects of CBD administration on the incidence of ischaemia- and reperfusion-induced arrhythmias, infarct size and platelet aggregation. In the control group, animals were given a bolus i.v. injection of vehicle (*n* = 19), via the right jugular vein, 10 min prior to CAO and a second bolus injection of vehicle 10 min prior to reperfusion. Preliminary studies in a small group of rats to determine doses of CBD to use in the I/R studies demonstrated that 50 µg·kg⁻¹ induced a small but significant depressor effect, while a lower dose of 10 µg·kg⁻¹ had no effect on MABP. We therefore selected these doses to determine whether a dose sufficient to induce a vascular response was required for any cardioprotective effect to be observed. Therefore, in the pre-ischaemia CBD-treated (CBD-PI) groups, animals were given a bolus i.v. dose of either 10 µg·kg⁻¹ (*n* = 5) or 50 µg·kg⁻¹ (*n* = 10), 10 min

prior to CAO and an additional bolus injection of vehicle 10 min prior to reperfusion. In the pre-reperfusion CBD-treated group (CBD-PR; *n* = 7), animals were given a bolus i.v. dose of vehicle, 10 min prior to CAO and an additional bolus injection of CBD (50 µg·kg⁻¹), 10 min prior to reperfusion. Because ischaemia itself induces both mast cell degranulation and platelet activation, we undertook a replicate series of experiments for the control and CBD (50 µg·kg⁻¹) pre-ischaemic treated protocols in sham-operated time controls (in which the ligature was placed around the left coronary artery but not tightened) to examine the direct effects of vehicle (*n* = 6) and CBD (50 µg·kg⁻¹; *n* = 9) on cardiac mast cell degranulation and collagen-induced platelet aggregation *ex vivo*.

Studies to investigate the pharmacological mechanism of CBD
In a separate group of rats we aimed to elucidate the type of receptors CBD acts on in the anaesthetized rat. Animals were anaesthetized and cannulated as previously described. MABP was measured via the carotid cannula and heart rate (HR) was calculated from the ECG. After surgery, rats were allowed to stabilize for 15 min before drug/vehicle administration. Post stabilization, animals were administered a bolus dose of vehicle followed subsequently (at regular intervals) by increasing doses of the proposed GPR55 agonist, O-1602 (5–100 ng·kg⁻¹; *n* = 3–8), firstly in the absence then presence of CBD (50 µg·kg⁻¹). To investigate the role of CBD at the CB₁ receptor, the haemodynamic effects of the CB₁ agonist, arachidonyl-2¢-chloroethylamide (ACEA; 3 mg·kg⁻¹; *n* = 4), were investigated in the absence and then presence of CBD (50 µg·kg⁻¹). To compare the effects of CBD on the ACEA-mediated vascular response with a known fatty acid amide hydrolase (FAAH) inhibitor, URB597 (1 mg·kg⁻¹; *n* = 4) was administered to rats prior to the administration of a bolus dose of ACEA (3 mg·kg⁻¹), as ACEA is thought to be susceptible to hydrolysis by FAAH.

In vitro *platelet aggregation studies.* To further investigate the anti-platelet effects of CBD an additional group of rats (*n* = 9) was killed by an overdose of pentobarbitone and blood collected via cardiac puncture into a tube containing heparin (final blood concentration of 20 U·mL⁻¹). Platelet aggregation was then determined by pre-incubating the blood with either vehicle or CBD (0.1–1000 µM) for 10 min prior to assessing platelet aggregation in response to collagen (5 µg·mL⁻¹).

Statistical analyses
For the haemodynamic data (expressed as mean ± SEM) Student's two-tailed *t*-test was used to compare pre-injection and post-injection MABP/HR values. One-way analysis of variance (ANOVA) and Dunnett's *post hoc* test was used to compare pre-occlusion and post-occlusion MABP/HR values. Post-occlusion MABP/HR comparisons between the control and CBD-treated groups were made using a two-way ANOVA and Bonferroni *post hoc* test. Ventricular and reperfusion arrhythmias were determined from the ECG trace and classified according to the Lambeth Conventions (Walker *et al.*, 1988). The effect of CBD on the number of ventricular ectopic beats

[VEBs; reported as singles, salvos, ventricular tachycardia (VT) and total VEB count and values expressed as mean ± SEM] was analysed using a one-way ANOVA and Dunnett's *post hoc* test. The effect of CBD on the incidence of VT, reversible and irreversible ventricular fibrillation (VF) and on mortality were analysed using Fisher's exact test. The effect of CBD treatment on both PR and QT intervals at various time points was investigated using a two-way ANOVA and Bonferroni *post hoc* test. The effects of CBD on infarct size, *ex vivo* and *in vitro* platelet aggregation, mast cell degranulation, and the effects of both CBD and URB597 on ACEA-induced vascular responses were analysed using Student's *t*-test or a one-way ANOVA and Dunnett's *post hoc* test, where appropriate.

Results

Effects of CBD on haemodynamic variables

The effects of CBD administration prior to and during CAO on MABP and HR are summarized in Table 1. Administration of 50 µg·kg⁻¹, but not 10 µg·kg⁻¹, CBD prior to CAO induced a significant but transient fall in MABP that reached a nadir 5 min post administration ($P < 0.05$). Treatment with CBD (50 µg·kg⁻¹), 10 min prior to reperfusion, similarly induced a significant but transient fall in MABP, which reached a nadir 11 min post administration ($P < 0.01$). All groups exhibited the characteristic fall in MABP upon occlusion of the coro-

nary artery ($P < 0.001$). Administration of CBD (50 µg·kg⁻¹) prior to reperfusion had no effect on the recovery of MABP post occlusion when compared with control animals. None of the CBD administration regimens had any significant effects on HR at any time point (Table 1).

Effect of CBD on I/R-induced ventricular arrhythmias

Induction of myocardial ischaemia resulted in the generation of a significant number of ventricular arrhythmias in the control group (Figure 1A). In most cases arrhythmias commenced 9–10 min post coronary occlusion in all groups (data not shown) and the majority occurred as VT (Figure 1A). Treatment with 50 µg·kg⁻¹, but not 10 µg·kg⁻¹, CBD prior to coronary occlusion significantly reduced the incidence of ischaemia-induced VEBs occurring as VT, and consequently the total number of VEBs compared with vehicle-treated animals (both $P < 0.001$; Figure 1A). Although CBD (50 µg·kg⁻¹) administration prior to coronary occlusion tended to reduce the incidence of reversible and total VF this did not achieve statistical significance (Figure 1B). The lower dose of CBD (10 µg·kg⁻¹) did not alter any type of VF compared with vehicle-treated animals. Further examination of the ECG revealed that myocardial ischaemia prolonged the QT interval in all groups but this was not significantly affected by CBD (50 µg·kg⁻¹) treatment (Figure 2A). Neither CAO nor CBD administration significantly altered the duration of the

Table 1 Summary of MABP and HR in rats given saline or CBD either prior to (time –10 min) ischaemia (performed at time 0 min), or prior to reperfusion (at +30 min)

Time (min)	Vehicle	CBD-PI (10 µg·kg⁻¹)	CBD-PI (50 µg·kg⁻¹)	CBD-PR (50 µg·kg⁻¹)
MABP (mmHg)				
–25	132 ± 3	119 ± 12	133 ± 4	127 ± 3
–10	132 ± 3	115 ± 8ᵃ	134 ± 4ᵃ	128 ± 2
–5	131 ± 3	113 ± 9	118 ± 5†	127 ± 3
0	128 ± 3	110 ± 10	132 ± 2	129 ± 3
1	87 ± 5***	79 ± 7***	90 ± 5***	97 ± 5***
3	94 ± 6***	89 ± 7**	97 ± 5***	108 ± 5**
20	105 ± 4**	80 ± 5***	112 ± 4**	112 ± 4*ᵇ
25	107 ± 4**	86 ± 8**	111 ± 3**	110 ± 3*
30	106 ± 4**	80 ± 9***	108 ± 3**	106 ± 2*
31	103 ± 4	89 ± 11	109 ± 8	86 ± 8††
35	109 ± 4	95 ± 12	116 ± 6	97 ± 5
60	110 ± 5	92 ± 13	118 ± 3	101 ± 5
150	98 ± 3	99 ± 7	112 ± 3	108 ± 4
HR (BPM)				
–25	438 ± 10	387 ± 35	404 ± 21	426 ± 13
–10	429 ± 8	412 ± 33ᵃ	392 ± 20ᵃ	429 ± 12
–5	431 ± 8	460 ± 35	396 ± 23	428 ± 15
0	429 ± 8	413 ± 48	401 ± 24	426 ± 15
1	429 ± 7	482 ± 26	406 ± 20	429 ± 13
3	425 ± 9	490 ± 48	404 ± 21	431 ± 13
20	401 ± 12	397 ± 52	383 ± 20	409 ± 15ᵇ
25	394 ± 9	430 ± 38	386 ± 20	418 ± 12
30	394 ± 9	445 ± 35	387 ± 22	411 ± 15
31	384 ± 9	464 ± 26	393 ± 22	418 ± 13
35	386 ± 9	476 ± 26	382 ± 27	406 ± 16
60	380 ± 10	420 ± 17	380 ± 23	414 ± 14
150	380 ± 11	426 ± 27	389 ± 18	416 ± 13

ᵃ,ᵇCBD administered.
†$P < 0.05$, ††$P < 0.01$ versus pre-injection value.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus pre-ischaemic value.
CBD, (-)-cannabidiol; CBD-PI, pre-ischaemia CBD-treated group; CBD-PR, pre-reperfusion CBD-treated group; HR, heart rate; MABP, mean arterial blood pressure.
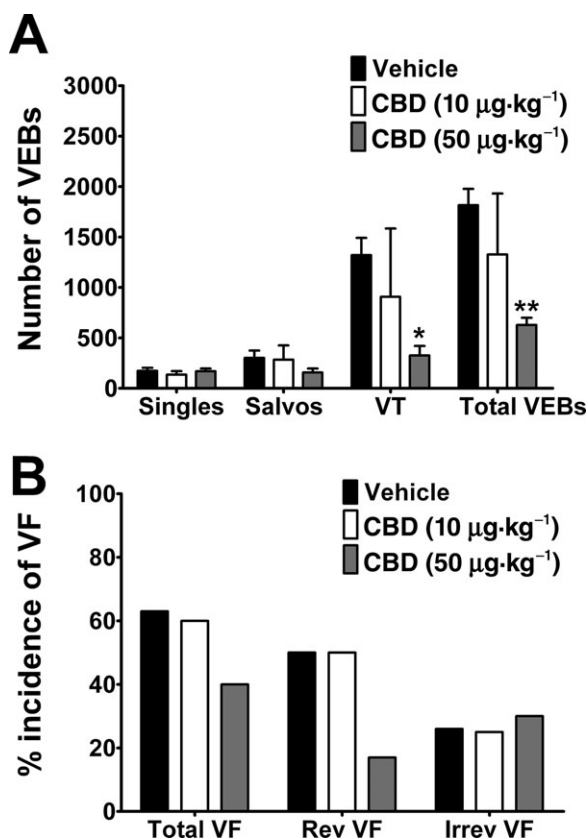
Figure 1 Effect of pre-ischaemic administration of CBD on (A) ischaemia-induced arrhythmias and (B) the incidence of VF. The incidence of each type of arrhythmia was recorded and the data expressed as mean $\pm$ SEM ($n = 4$–14). *$P < 0.05$, **$P < 0.01$ versus vehicle. The incidence of each type of VF was recorded and the data expressed as the mean ($n = 5$–19). CBD, (-)-cannabidiol; VEB, ventricular ectopic beat; VF, ventricular fibrillation; VT, ventricular tachycardia.
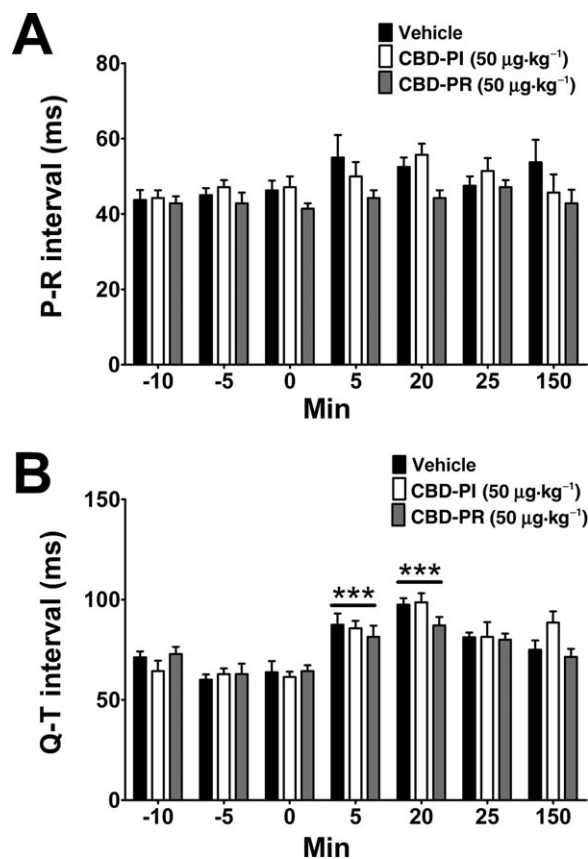

Figure 2 Effect of CBD administration on (A) PR and (B) QT intervals. Both PR and QT intervals were measured from the ECG in milliseconds (ms) and the data expressed as mean $\pm$ SEM ($n = 7$–8). ***$P < 0.001$ versus pre-ischaemic values. CBD, (-)-cannabidiol; CBD-PI, pre-ischaemia CBD-treated group; CBD-PR, pre-reperfusion CBD-treated group.
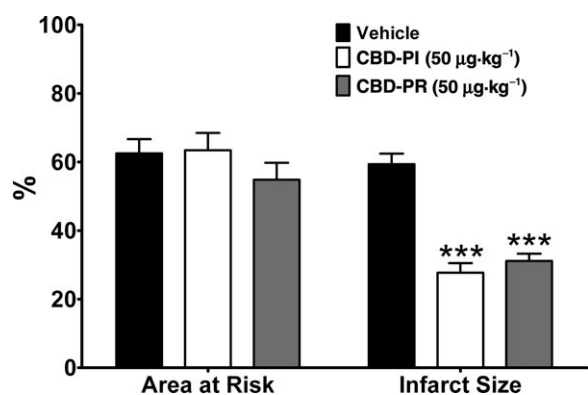
PR interval in any of the groups examined (Figure 2B). Reperfusion of the myocardium resulted in the generation of ventricular arrhythmias, the majority occurring as VT. Treatment with CBD (50 μg·kg⁻¹) immediately prior to reperfusion did not affect the occurrence of VEBs when compared with the vehicle control [77 $\pm$ 50 vs. 92 $\pm$ 45 (total VEBs); not significant]; neither control nor CBD (50 μg·kg⁻¹)-treated groups experienced any VF during reperfusion.

Effect of CBD on infarct size
Figure 3 illustrates the effects of the higher dose of CBD (50 μg·kg⁻¹) on both area at risk (percentage of left ventricular area) and infarct size (percentage of area at risk). Area at risk was similar across all groups. Administration of CBD (50 μg·kg⁻¹) prior to coronary occlusion significantly reduced infarct size, as did its administration immediately prior to reperfusion, when compared with vehicle-treated control rats (both $P < 0.001$; Figure 3).

Effect of CBD on platelet aggregation
In time-matched sham-operated rats CBD (50 μg·kg⁻¹) significantly reduced collagen-induced platelet aggregation *ex vivo*

Figure 3 Effect of CBD administered both prior to ischaemia and prior to reperfusion, on area at risk and infarct size. Area at risk was measured as a percentage of total left ventricular area and infarct size was measured as a percentage of area at risk. Both sets of data are expressed as the mean $\pm$ SEM ($n = 7$–8). ***$P < 0.001$ versus vehicle. CBD, (-)-cannabidiol; CBD-PI, pre-ischaemia CBD-treated group; CBD-PR, pre-reperfusion CBD-treated group.

compared with vehicle-treated sham-operated rats ($P < 0.05$; Figure 4A). Administration of CBD (50 μg·kg⁻¹) prior to ischaemia similarly attenuated collagen-induced platelet aggregation measured *ex vivo* ($P < 0.05$; Figure 4A).
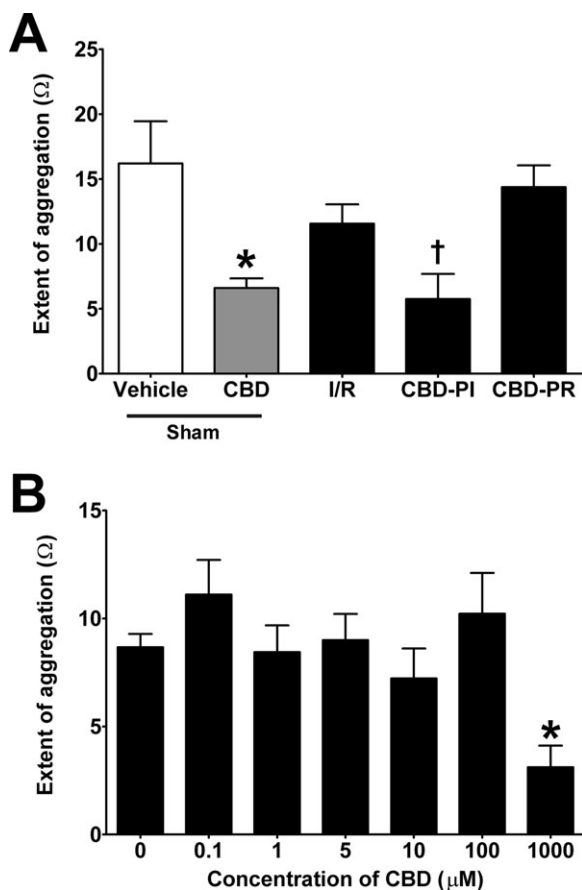
Figure 4 Effect of CBD (50 μg·kg^{-1}) treatment on (A) *ex vivo* and (B) *in vitro* platelet aggregation in response to collagen (5 μg·mL^{-1}). Platelet aggregation was expressed in terms of ohms (Ω) and expressed as the mean $\pm$ SEM ($n = 6$–9). *$P < 0.05$ versus vehicle; †$P < 0.05$ versus I/R. CBD, (-)-cannabidiol; CBD-PI, pre-ischaemia CBD-treated group; CBD-PR, pre-reperfusion CBD-treated group; I/R, ischaemia/reperfusion.

Interestingly, when CBD (50 μg·kg^{-1}) was administered immediately prior to reperfusion it did not significantly affect platelet aggregation when compared with the control. In a series of experiments to investigate the *in vitro* effects of CBD on agonist-induced platelet aggregation only the highest concentration of CBD investigated (1 mM) significantly attenuated collagen-induced platelet aggregation compared with the vehicle ($P < 0.05$; Figure 4B).

Effect of CBD on I/R-induced cardiac mast cell degranulation
Figure 5 summarizes the effects of CBD on cardiac mast cell degranulation. In vehicle-treated sham-operated animals, approximately 44% of cardiac mast cells were degranulated and similar numbers were found in sham-operated rats given CBD (50 μg·kg^{-1}). Myocardial I/R induced significant ($P < 0.001$) mast cell degranulation in vehicle-treated control rats, when compared with the vehicle sham-operated group; and administration of CBD (50 μg·kg^{-1}) either prior to or post CAO did not alter the extent of mast cell degranulation induced by I/R alone.

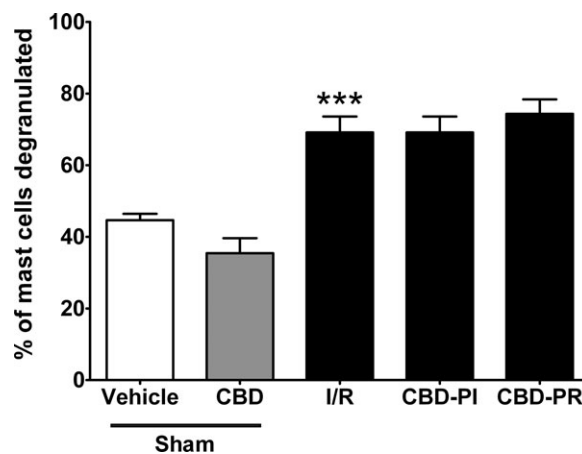**Figure 5** Effects of CBD (50 μg·kg^{-1}) and I/R on the percentage of mast cells degranulated in the rat myocardium. Mast cell degranulation was measured as the percentage of the total number of mast cells present that had undergone degranulation and is expressed as the mean $\pm$ SEM ($n = 6$–9). The percentage incidence of mast cell degranulation was determined at a magnification of ×400 and encompassed an entire cross-section of ventricular tissue. Both sham-operated and I/R animals were treated with a bolus dose of either vehicle or CBD. The effect of I/R alone on mast cell degranulation was determined via a comparison of vehicle-treated I/R animals with vehicle-treated sham-operated animals. ***$P < 0.001$ versus vehicle sham-operated. CBD, (-)-cannabidiol; CBD-PI, pre-ischaemia CBD-treated group; CBD-PR, pre-reperfusion CBD-treated group; I/R, ischaemia/reperfusion.

Receptor-mediated effects of CBD
The haemodynamic effects of a range of doses (5–100 ng·kg^{-1}) of O-1602 (GPR55 agonist) were examined; however, no reproducible measurable depressor response was obtained over the dose range tested (data not shown). Administration of the CB$_1$ receptor agonist, ACEA (3 mg·kg^{-1}), induced a depressor response that was unaffected by pretreatment with CBD (50 μg·kg^{-1}; Figure 6), a proposed CB$_1$ antagonist. Furthermore, a similar ACEA-induced depressor response, to that observed in the presence of CBD, was demonstrated when ACEA was administered in the presence of the selective FAAH inhibitor, URB597 (1 mg·kg^{-1}; Figure 6).

Discussion

Previous studies have demonstrated that prolonged administration of CBD exerts neuroprotective and cardioprotective effects that involve anti-inflammatory, antioxidant and anti-necrotic actions of the compounds (reviewed by Mechoulam *et al.*, 2007). The present study is the first to demonstrate that in the setting of myocardial I/R CBD can provide acute cardioprotection, in that it both suppresses ischaemia-induced ventricular arrhythmias and attenuates infarct size when given immediately prior to ischaemia onset. Moreover, and potentially more clinically relevant, CBD also reduces infarct size when given at the time of reperfusion. These findings imply that the anti-arrhythmic and cytoprotective effects of CBD are achieved through different mechanisms.

Anti-arrhythmic effects of CBD
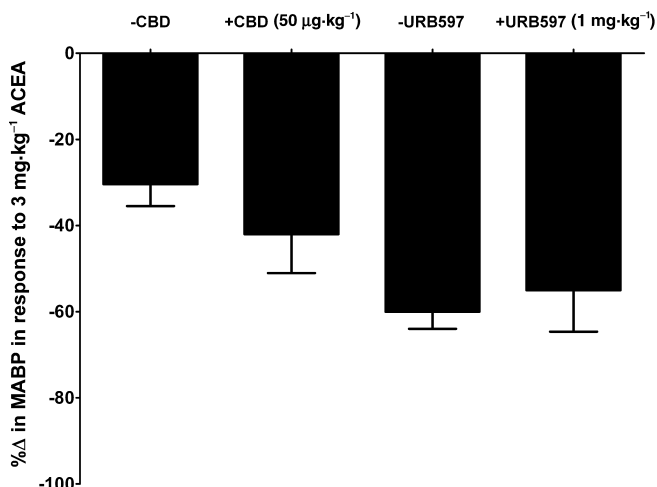There are several explanations for the mechanisms underlying the anti-arrhythmic effect of CBD, one of which could be

Figure 6 Receptor-mediated effects of (-)-cannabidiol (CBD). The role of CBD as either a CB_1 antagonist or potential fatty acid amide hydrolase (FAAH) inhibitor was investigated by comparing the effects of CBD (50 µg·kg⁻¹) and the selective FAAH inhibitor, URB597 (1 mg·kg⁻¹), on arachidonyl-2¢-chloroethylamide (ACEA) (3 mg·kg⁻¹)-mediated vascular responses. Agonist-induced changes in mean arterial blood pressure (MABP) were recorded and expressed as a percentage change in MABP (%Δ; $n = 4$ for each treatment).

a direct electrophysiological effect. CBD has been reported to inhibit the slow component of the delayed rectifying potassium channel (IKs) in ventricular myocytes (Mamas and Terrar, 1998). IKs blockers prolong cardiac action potential duration and QT interval and suppress electrically induced arrhythmias in the presence of myocardial ischaemia (Tamargo *et al.*, 2004). However ECG analysis revealed that CBD did not prolong QT interval before ischaemia, nor did it further enhance the ischaemia-induced QT prolongation, suggesting that this is an unlikely explanation for CBD's anti-arrhythmic effects.

The finding that CBD inhibits collagen-induced platelet aggregation *ex vivo* suggests an alternative mechanism for its anti-arrhythmic effect, as numerous studies have shown that anti-platelet agents are anti-arrhythmic by virtue of their ability to prevent release of arrhythmogenic substances such as thromboxane A2 and 5-hydroxytryptamine (Wainwright *et al.*, 1988; Barnes and Coker, 1995). What is interesting, however, is that CBD only inhibited platelet aggregation *ex vivo* when given to sham-operated animals or prior to ischaemia, but not when given prior to reperfusion. While this finding supports the notion that an effect on platelets may be responsible for its anti-arrhythmic effect during ischaemia but not following reperfusion, it cannot explain the ability of CBD to preserve tissue from cell death. Moreover, what this observation may also suggest is that, the mechanism by which CBD inhibits collagen-induced platelet aggregation when administered under physiological conditions (i.e. in sham-operated and pre-ischaemia) is somehow absent or abrogated under ischaemic conditions (i.e. administered prior to reperfusion). In addition, as data from the *in vitro* studies demonstrated that CBD (in micromolar concentrations) did not affect platelet aggregation, this may further support the idea that CBD only modulates platelet aggregation through interference with an endogenous system and not directly.

While there is no immediate explanation for this, recent studies have demonstrated that platelets express both CB_1 and CB_2 receptors (Deusch *et al.*, 2004) and that the endocannabinoids anandamide (Maccarrone *et al.*, 1999) and 2-arachidonoylglycerol (2-AG; Baldassarri *et al.*, 2008) both induce platelet activation/aggregation, although whether or not through CB_1 and/or CB_2 receptor activation remains controversial. Studies have demonstrated that levels of 2-AG are increased in the ischaemically preconditioned heart (Wagner *et al.*, 2006), thus ischaemia-induced elevated levels of 2-AG may contribute to platelet activation by abrogating the anti-platelet effects of CBD via competition for the same receptors. However, further studies to investigate the various effects of endocannabinoids within the ischaemic myocardium are clearly required.

A third explanation for the anti-arrhythmic effect of CBD is through an action on mast cells, as previous studies have demonstrated that CBD induces mucosal mast cell degranulation (Giudice *et al.*, 2007). Treatment with mast cell degranulating agents prior to ischaemia has been shown to elicit a profound anti-arrhythmic effect via the depletion of mast cell-derived cytotoxic compounds (Parikh and Singh, 1997; Walsh *et al.*, 2009a). However, in the present study it was demonstrated that CBD does not induce cardiac mast cell degranulation, as shown by the lack of effect in hearts from rats subjected to sham treatment. Moreover, CBD did not prevent ischaemia-induced mast cell degranulation, a strategy that has also been demonstrated to be cardioprotective (Humphreys *et al.*, 1998; Walsh *et al.*, 2009b). Taken together this evidence does not support the involvement of a cardiac mast cell-dependent pathway in the anti-arrhythmic effects of CBD.

Although we did not investigate this in the current study, CBD may also mediate its anti-arrhythmic effects through modulation of one or more endogenous cardioprotective agents that have demonstrated anti-arrhythmic effects, including anandamide (Ugdyzhekova *et al.*, 2001; Krylatov *et al.*, 2002; Hajrasouliha *et al.*, 2008). In a previous study, CBD (10–20 µM) was shown to inhibit both the anandamide membrane transporter (thus preventing cellular uptake) and FAAH (thus preventing hydrolysis of anandamide) (Bisogno *et al.*, 2001), both of which would elevate endogenous anandamide levels. In the present study, we attempted to determine whether or not CBD behaved in a similar way to the selective FAAH inhibitor, URB597, by assessing their ability to enhance the vascular response to ACEA, which has been shown to be susceptible to FAAH hydrolysis. However, neither URB597 nor CBD augmented the response to ACEA, therefore the question as to whether or not CBD is acting via inhibition of endocannabinoid breakdown remains to be answered. Moreover, whether or not the estimated low plasma concentration of CBD (~2 µM) achieved in the myocardial I/R study was sufficient to increase endogenous anandamide levels also remains to be determined.

Infarct sparing effect of CBD
In relation to the infarct sparing effect, CBD has previously been shown to protect against both cerebral (Mishima *et al.*, 2005; Hayakawa *et al.*, 2007) and myocardial I/R injury (Durst

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et al., 2007) and evidence points to this being achieved through a direct anti-inflammatory effect (Weiss et al., 2008) mediated by CB_2 receptors (Hajrasouliha et al., 2008). Our data agree with the findings of Durst et al. (2007) in that CBD significantly reduces tissue injury; however, our study significantly extends their observations in two ways. First, the study by Durst's group involved both prolonged (7 day) CBD administration and a much later time point for assessment of tissue injury (i.e. at a time when the key pathological events are inflammation and scar formation), whereas we have assessed tissue injury at a time when immediate lethal injury has occurred (within 2 h of reperfusion) but delayed injury has not yet begun. Thus our data show that CBD can undoubtedly reduce the initial injury that is associated with rapid events such as oxidative stress and activation of death signalling pathways (Logue et al., 2005). Second, we have also shown that CBD can do this when given just before restoration of blood flow, implying a potentially valuable clinical application in patients undergoing clinical reperfusion.

Quite how CBD exerts cardioprotection against immediate lethal injury has yet to be fully explored. One suggestion is that CBD may act as a peroxisome proliferator-activated receptor gamma agonist (O'Sullivan et al., 2009), activation of that has previously been shown to reduce infarct size in a murine model of myocardial I/R via a profound anti-inflammatory effect (Honda et al., 2008). In addition, CBD may confer tissue protection by acting as a CB_1 receptor antagonist resulting in preferential activation of CB_2 receptors by endocannabinoids, as the bulk of evidence points to endocannabinoids reducing infarct size via activation of CB_2 rather than CB_1 receptors (Hajrasouliha et al., 2008; Lim et al., 2009). This is in contrast to the effects of synthetic CB_1 or CB_2 receptor agonists, neither of which reduce infarct size (Underdown et al., 2005), suggesting that endocannabinoid-induced protection may be mediated by receptors other than the typical CB_1/CB_2 receptors. In support of the latter, our own study demonstrated that CBD does not prevent ACEA-induced hypotension, suggesting that under the present conditions, CBD (at a dose of 50 $\mu g \cdot kg^{-1}$) does not act as a CB_1 receptor antagonist. It could act as an antagonist at the orphan receptor GPR55, which has been proposed as a third cannabinoid receptor (Ryberg et al., 2007), through inhibition of a detrimental effect of anandamide action at this receptor. However, data from the present study suggest that GPR55 receptors are not present on the rat vasculature (due to a lack of observed haemodynamic effects of the GPR55 agonist, O-1602), although this does not rule out the presence of these receptors in the myocardium. To date there are no studies that have explored the role of GPR55 in the setting of acute myocardial I/R, although this clearly would be of value.

Rather than acting through a receptor, CBD may induce a tissue sparing effect through a direct action on ion channels. A very recent study (Ryan et al., 2009) has shown that the neuroprotective effect of CBD may be a result of restoration of intracellular Ca^{2+} homeostasis at the level of the mitochondria; using hippocampal slices this group found that under normal physiological conditions CBD had minimal effects on mitochondrial calcium mobilization, while under conditions of high extracellular K^+ it significantly reduced cytosolic Ca^{2+} concentration. This effect was abolished by inhibition of the mitochondrial Na^+/Ca^{2+} exchanger (NCX), but not via an inhibitor of the mitochondrial permeability transition pore (mPTP), suggesting that, under pathophysiological conditions, CBD improves intracellular Ca^{2+} homeostasis through modulation of NCX activity. A similar effect on the cardiomyocyte mitochondria would therefore be expected to help prevent calcium overload, one of the key mechanisms of immediate lethal injury following reperfusion. In addition, anandamide has recently been shown to reduce inositol-1,4,5,-trisphosphate receptor (IP_3R)-mediated nuclear Ca^{2+} release in cardiomyocyte nuclear envelopes expressing both CB_1 and CB_2 receptors (Currie et al., 2008). This effect was significantly attenuated by both CB_1 and CB_2 receptor antagonists, providing the first evidence for a nuclear receptor site of action for cannabinoids in cardiomyocytes. Further study of a cardioprotective role for CBD, mediated at either the mitochondrial or nuclear level, is therefore clearly warranted.

In summary, to our knowledge this is the first study to demonstrate an anti-arrhythmic effect of CBD following myocardial I/R. This study is also the first to demonstrate that acute administration of a single dose of CBD is sufficient to reduce myocardial tissue injury irrespective of whether it is administered prior to or post coronary occlusion. While further detailed studies are required to elucidate the mechanism by which CBD preserves tissue in I/R, these data expand on the currently very limited literature detailing the role of CBD in the cardiovascular system and firmly establishes its potential as a cardioprotective agent.

Statement of conflict of interests

None.

References

Baldassarri S, Bertoni A, Bagarotti A, Sarasso C, Zanfa M, Catani MV et al. (2008). The endocannabinoid 2-arachidonoylglycerol activates human platelets through non-CB1/CB2 receptors. J Thromb Haemost 6: 1772–1779.

Barnes CS, Coker SJ (1995). Failure of nitric oxide donors to alter arrhythmias induced by acute myocardial ischaemia or reperfusion in anaethetized rats. Br J Pharmacol 114: 349–356.

Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I et al. (2001). Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br J Pharmacol 134: 845–852.

Clark C, Foreman MI, Kane KA, McDonald FM, Parratt JR (1980). Coronary artery ligation in anesthetized rats as a method for the production of experimental dysrrhythmias and for the determination of infarct size. J Pharmacol Methods 3: 357–368.

Currie S, Rainbow RD, Ewart MA, Kitson S, Herradon-Pliego E, Kane KA et al. (2008). IP3R-mediated Ca2+ release is modulated by anandamide in isolated cardiac nuclei. J Mol Cell Cardiol 45: 804–811.

Deusch E, Kress HG, Kraft B, Kozek-Langenecker SA (2004). The procoagulatory effects of delta-9-tetrahydrocannabinol in human platelets. Anesth Analg 99: 1127–1130.

Durst R, Danenberg H, Gallily R, Beeri R, Mechoulam R, Meir K et al. (2007). Cannabidiol, a non-psychoactive Cannabis constituent

protects against myocardial ischemic reperfusion injury. *Am J Physiol Heart Circ Physiol* **293**: 3602–3607.

Flores NA, Goulielmos NV, Seghatchian MJ, Sheridan DJ (1994). Myocardial ischaemia induces platelet activation with adverse electrophysiological and arrhythmogenic effects. *Cardiovasc Res* **28**: 1662–1671.

Formukong EA, Evans AT, Evans FJ (1989). The inhibitory effects of cannabinoids, the active constituents of *Cannabis sativa* L. on human and rabbit platelet aggregation. *J Pharm Pharmacol* **41**: 705709.

Giudice ED, Rinaldi L, Passarotto M, Facchinetti F, D'Arrigo A, Guiotto A *et al*. (2007). Cannabidiol, unlike synthetic cannbinoids, triggers activation of RBL-2H3 mast cells. *J Leukoc Biol* **81**: 1512–1522.

Hajrasouliha AR, Tavakoli S, Ghasemi M, Jabehdar-Maralani P, Sadeghipour H, Ebrahimi F *et al*. (2008). Endogenous cannabinoids contribute to remote ischemic preconditioning via cannabinoid CB_2 receptors in the rat heart. *Eur J Pharmacol* **579**: 246–252.

Hampson AJ, Grimaldi M, Axelrod J, Wink D (1998). Cannabidiol and (-)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc Natl Acad Sci USA* **95**: 8268–8273.

Hayakawa K, Mishima K, Nozako M, Ogata A, Hazekawa M, Liu AX *et al*. (2007). Repeated treatment with cannabidiol but not Delta9-tetrahydrocannabinol has a neuroprotective effect without the development of tolerance. *Neuropharmacology* **52**: 1079–1087.

Honda T, Kaikita K, Tsujita K, Hayasaki T, Matsukawa M, Fuchigami S *et al*. (2008). Pioglitazone, a peroxisome proliferator-activated receptor-gamma agonist, attenuates myocardial ischemia-reperfusion injury in mice with metabolic disorders. *J Mol Cell Cardiol* **44**: 915–926.

Humphreys RA, Kane KA, Parratt JR (1998). Lack of involvement of mast cell degranulation in the antiarrhythmic effect of preconditioning in rats. *J Cardiovasc Pharmacol* **31**: 418–423.

Krylatov AV, Uzhachenko RV, Maslov LN, Bernatskaya NA, Makriyannis A, Mechoulam R *et al*. (2002). Endogenous cannabinoids improve myocardial resistance to arrhythmogenic effects of coronary occlusion and reperfusion: A possible mechanism. *Bull Exp Biol Med* **133**: 144–147.

Lim SY, Davidson SM, Yellon DM, Smith CC (2009). The cannabinoid CB1 receptor antagonist, rimonabant, protects against acute myocardial infarction. *Basic Res Cardiol* **104**: 781–792.

Logue SE, Gustafsson AS, Samali A, Gottlieb RA (2005). Ischemia/reperfusion injury at the intersection with cell death. *J Moll Cell Cardiol* **38**: 21–33.

Maccarrone M, Bari M, Menichelli A, Del Prinipe D, Agro AF (1999). Anandamide activates human platelets through a pathway independent of the arachidonate cascade. *FEBS Lett* **447**: 277–282.

Malfait AM, Gallily R, Sumariwalla PF, Malik AS, Andreakos E, Mechoulam R *et al*. (2000). The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. *Proc Natl Acad Sci USA* **97**: 9561–9566.

Mamas MA, Terrar DA (1998). Differential sensitivity to cannabidiol of the two components of delayed rectifier potassium currents in guinea pig isolated ventricular myocytes. *Br J Pharmacol* **123**: 319P.

Mechoulam R, Peters M, Murillo-Rodriguez E, Hanus LO (2007). Cannabidiol – recent advances. *Chem Biodivers* **4**: 1678–1692.

Messina A, Knight KR, Dowsing BJ, Zhang B, Phan LH, Hurley JV *et al*. (2000). Localization of inducible nitric oxide synthase to mast cells during ischemia/reperfusion injury of skeletal muscle. *Lab Invest* **80**: 423–431.

Mishima K, Hayakawa K, Abe K, Ikeda T, Egashira N, Iwasaki K *et al*. (2005). Cannabidiol prevents cerebral infarction via a serotonergic 5-hydroxytryptamine1A receptor-dependent mechanism. *Stroke* **36**: 1077–1082.

O'Sullivan SE, Sun Y, Bennett AJ, Randall MD, Kendall DA (2009). Time-dependent vascular effects of cannabidiol in the rat aorta. *Eur J Pharmacol* **612**: 61–68.

Parikh V, Singh M (1997). Resident cardiac mast cells and the cardioprotective effect of ischemic preconditioning in isolated rat heart. *J Cardiovasc Pharmacol* **30**: 149–156.

Pertwee RG (2008). The diverse CB_1 and CB_2 receptor pharmacology of three plant cannabinoids: Δ^9-tetrahydrocannabinol, cannabidiol and Δ^9-tetrahydrocannabivarin. *Br J Pharmacol* **153**: 199–215.

Rakhshan F, Day TA, Blakely RD, Barker EL (2000). Carrier-mediated uptake of the endogenous cannabinoid anandamide in RBL-2H3 cells. *J Pharmacol Exp Ther* **292**: 960–967.

Russo EB, Burnett A, Hall B, Parker KK (2005). Agnostic properties of cannabidiol at 5-HT1a receptors. *Neurochem Res* **30**: 1037–1043.

Ryan D, Drysdale AJ, Lafourcade C, Pertwee RG, Platt B (2009). Cannabidiol targets mitochondria to regulate intracellular Ca^{2+} levels. *J Neurosci* **29**: 2053–2063.

Ryberg E, Larsson N, Sjögren S, Hjorth S, Hermansson NO, Leonova J *et al*. (2007). The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* **152**: 1092–1101.

Tamargo J, Caballero R, Gomez R, Valenzuela C, Delpon E (2004). Pharmacology of cardiac potassium channels. *Cardiovasc Res* **62**: 9–33.

Thomas A, Baillie GL, Phillips AM, Razdan RK, Ross RA, Pertwee RG (2007). Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists *in vitro*. *Br J Pharmacol* **150**: 613–623.

Ugdyzhekova DS, Bernatskaya NA, Stefano JB, Graier VF, Tam SW, Mechoulam R (2001). Endogenous cannabinoid anandamide increases heart resistance to arrhythmogenic effects of epinephrine: role of CB_1 and CB_2 receptors. *Bull Exp Biol Med* **131**: 300–302.

Underdown NJ, Hiley CR, Ford WR (2005). Anandamide reduces infarct size in rat isolated hearts subjected to ischaemia-reperfusion by a novel cannabinoid mechanism. *Br J Pharmacol* **146**: 809–816.

Vannacci A, Giannini L, Passani MB, Di Felice A, Pierpaoli S, Zagli G *et al*. (2004). The endocannabinoid 2-arachidonylglycerol decreases the immunological activation of guinea pig mast cells: involvement of nitric oxide and eicosanoids. *J Pharmacol Exp Ther* **311**: 256–264.

Wagner JA, Abesser M, Harvery-White J, Ertl G (2006). 2-Arachidonylglycerol acting on CB1 cannabinoid receptors mediates delayed cardioprotection induced by nitric oxide in rat isolated hearts. *J Cardiovasc Pharmacol* **47**: 650–655.

Wainwright CL, Parratt JR, Bigaud M (1988). The effects of PAF antagonists on ischaemia and reperfusion arrhythmias and ischaemia-induced platelet aggregation. *Biomed Biochim Acta* **47**: 224–227.

Walker MJA, Curtis MJ, Hearse DJ, Campbell RWF, Janse MJ, Yellon DM *et al*. (1988). The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia, infarction, and reperfusion. *Cardiovasc Res* **22**: 447–455.

Walsh SK, Kane KA, Wainwright CL (2009a). Mast cell degranulation – a mechanism for the anti-arrhythmic effect of endothelin-1? *Br J Pharmacol* **157**: 716–723.

Walsh SK, Kane KA, Wainwright CL (2009b). Mast cells, peptides and cardioprotection – an unlikely marriage? *Auton Autacoid Pharmacol* **29**: 73–84.

Weiss L, Zeira M, Reich S, Slavin S, Raz I, Mechoulam R *et al*. (2008). Cannabidiol arrests onset of autoimmune diabetes in NOD mice. *Neuropharmacology* **54**: 244–249.