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The cannabinomimetic arachidonyl-2-chloroethylamide (ACEA) acts on capsaicin-sensitive $TRPV_1$ receptors but not cannabinoid receptors in rat joints

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1 The vasoactive effects of the synthetic cannabinoid (CB) arachidonyl-2-chloroethylamide (ACEA) was tested in the knee joints of urethane-anaesthetised rats. Experiments were also performed to determine whether these vasomotor responses could be blocked by the selective CB_1 receptor antagonists AM251 (*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) (10^{-9} mol) and AM281 (1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-4-morpholinyl-1*H*-pyrazole-3-carboxamide) (10^{-8} mol), as well as the selective CB_2 receptor antagonist AM630 (6-iodo-2-methyl-1-[2-4(morpholinyl)ethyl]-[1*H*-indol-3-yl](4-methoxyphenyl)methanone) (10^{-8} mol).

2 Peripheral application of ACEA (10^{-14}–10^{-9} mol) onto the exposed surface of the knee joint capsule caused a dose-dependent increase in synovial blood flow. The dilator action of the CB occurred within 1 min after drug administration and rapidly returned to control levels shortly thereafter. The maximal vasodilator effect of ACEA corresponded to a 30% increase in articular perfusion compared to control levels.

3 The hyperaemic action of ACEA was not significantly altered by coadministration of AM251, AM281 or AM630 ($P>0.05$; two-way ANOVA).

4 The transient receptor potential channel vanilloid receptor 1 ($TRPV_1$) antagonist capsazepine (10^{-6} mol) significantly reduced the vasodilator effect of ACEA on joint blood vessels ($P=0.002$). Furthermore, destruction of unmyelinated and thinly myelinated joint sensory nerves by capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) treatment also attenuated ACEA responses ($P<0.0005$).

5 These data clearly demonstrate a vasodilator effect of the cannabinomimetic ACEA on knee joint perfusion. Rather than a classic CB receptor pathway, ACEA exerts its vasomotor influence by acting *via* $TRPV_1$ receptors located on the terminal branches of capsaicin-sensitive afferent nerves innervating the joint.
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Abbreviations: ACEA, arachidonyl-2-chloroethylamide; CB, cannabinoid; DMSO, dimethylsulphoxide; LDI, Laser Doppler perfusion Imager; MAP, mean arterial pressure; PU, perfusion units; $TRPV_1$, transient receptor potential channel vanilloid receptor 1

Introduction

Cannabinoids (CBs) are a group of synthetic alkaloids derived from the hemp plant *Cannabis sativa*, which can exert potent pharmacological effects on the cardiovascular and nervous systems. Recently, endogenous CBs have been identified highlighting a novel and enticing area of research into their physiological roles in tissue function. The first of these so-called endocannabinoids to be described was anandamide (Devane *et al*., 1992), which is biosynthesised from cell membranes and released locally to act on neighbouring cells in an autocrine or paracrine manner. Anandamide has been shown to be widely distributed in the central and peripheral nervous systems of the rat (Felder *et al*., 1996) where one of its main functions is to inhibit nociception and pain perception (for reviews see Pertwee, 2001; Rice *et al*., 2003).

In addition to their analgesic effects, CBs can also act on the cardiovascular system to cause hypotension and bradycardia (Varga *et al*., 1996; Jarai *et al*., 1999; Högestatt & Zygmunt, 2002). A number of *in vitro* and *in vivo* studies have convincingly shown that local administration of CBs causes vasodilatation in the microvasculature of the heart (Wagner *et al*., 2001; Ford *et al*., 2002), brain (Ellis *et al*., 1995; Wagner *et al*., 2001), liver (Garcia *et al*., 2001) and mesentery (Randall *et al*., 1996; White & Hiley, 1998; Jarai *et al*., 1999). It was originally thought that the dilator effect of CBs was due to prejunctional inhibition of the sympathetic nerve terminals, which innervate blood vessels leading to a decline in noradrenergic vasoconstrictor tone (Varga *et al*., 1995; 1996). Later studies refute this mechanism by demonstrating a lack of effect of anandamide and synthetic CBs on noradrenaline release and vasoconstrictor responses following electrical stimulation of perivascular sympathetic nerves (Malinowska

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et al., 1997; Lay *et al*., 2000). Opposing evidence has also been described in other reports wherein the hypotensive effects of the synthetic CB HU210 are unaltered by chemical sympathetic blockade (Vidrio *et al*., 1996; Wagner *et al*., 2001). An alternative explanation for CB-mediated vasodilatation has been proposed, which involves the peripheral release of potent dilator neuropeptides from sensory nerves. Zygmunt *et al*. (1999) found that in an isolated artery preparation, anandamide activated perivascular afferents to cause the peripheral release of the vasodilator neuropeptide calcitonin gene-related peptide. Other reports support this observation by showing that the hypotensive and dilator action of CBs is lost following chemical degeneration of unmyelinated sensory nerve fibres with the selective neurotoxic agent capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) (Ralevic *et al*., 2000; Smith & McQueen, 2001). Thus, endocannabinoids appear to act on capsaicin-sensitive afferents to promote neurogenic inflammation.

To date, two distinct CB receptor subtypes have been identified and cloned, *viz* CB_1 (Matsuda *et al*., 1990) and CB_2 (Munro *et al*., 1993). CB_1 receptors have been localised throughout the central nervous system as well as on peripheral nerve terminals where their activation modulates neurotransmitter release (Tsou *et al*., 1998; Pertwee, 1999; Farquhar-Smith *et al*., 2000). In contrast, CB_2 receptors are believed to be primarily located on immunocytes, although there is some evidence to suggest that these receptors are also expressed by neuronal tissue (Griffin *et al*., 1997; Zhang *et al*., 2003). Anandamide has been shown to be a full agonist at the CB_1 receptor with a weak affinity for CB_2 receptors (Pertwee, 1999); however, more intriguingly anandamide is also a full agonist at the capsaicin-binding vanilloid receptor $TRPV_1$ (transient receptor potential channel vanilloid receptor 1) (Zygmunt *et al*., 1999; Smart *et al*., 2000). In rat knee joints, anandamide sensitises peripheral nociceptors through a $TRPV_1$-dependent pathway (Gauldie *et al*., 2001); however, the vasomotor corollary of CBs in articular tissues is currently unknown. This study examined the *in vivo* effects of the synthetic anandamide analogue arachidonyl-2-chloroethylamide (ACEA) on knee joint blood flow and characterised the receptor subtype responsible for these vasomotor responses.

Methods

Adult male Wistar rats (240–430 g) were caged in pairs at room temperature under a 12/12 h (07:00/19:00) light–dark cycle and fed *ad libitum* with standard rodent food. On the day of blood flow assessment, animals were anaesthetised with urethane ($1.5\,mg\,kg^{-1}$, i.p.) and depth of anaesthesia was confirmed by an absence of the pedal withdrawal reflex. The hindlimb of the rat was shaved and the animal was then placed in dorsal recumbency on a thermostatically controlled heating pad (Fine Science Tools Inc., Vancouver, Canada) to maintain a core body temperature of 37°C. All subsequent surgical and experimental procedures had received prior approval from the University of Calgary Animal Care Committee, which is in accordance with the Canadian Council for Animal Care guidelines.

Surgical preparation

The fur covering the ventral aspect of the rat neck was lacquered using mineral oil and a longitudinal incision was made in the skin to expose the trachea, which was cannulated to allow unrestricted breathing. The left carotid artery was then isolated and cannulated with a polyethylene catheter (0.5 mm internal diameter, 1.0 mm outer diameter; Portex, Kent, U.K.) containing heparinised saline ($50\,U\,100\,ml^{-1}$). This carotid cannula was connected in series to a pressure transducer (Stoelting Co, Illinois, U.S.A.) and mean arterial pressure (MAP) was continuously recorded on a differentially amplified blood pressure monitor (World Precision Instruments, Florida, U.S.A.). The skin covering the anteromedial region of the rat stifle (knee) joint was excised and all superficial fascia removed to allow unrestricted access to joint capsular blood vessels. Following joint exposure, warmed (37°C) physiological saline (0.9% NaCl) was intermittently superfused over the surface of the knee to prevent tissue desiccation. A number of studies have consistently shown that topical application of saline in this manner has no discernible effect on joint blood flow (McDougall *et al*., 1997; Barin & McDougall 2003; McDougall, 2003).

Blood flow assessment of the rat knee

Blood perfusion of the rat knee was measured using a Laser Doppler perfusion Imager (LDI) (Moor Instruments Ltd, Axminster, U.K.), which detects relative changes in tissue blood flow. The protocol for articular perfusion measurement has been described in more detail elsewhere (Karimian *et al*., 1995; McDougall *et al*., 1995), but briefly involves the scanning of the exposed joint with a low-power (2 mW) red laser beam (wavelength = 633 nm). By carefully placing black cloth around the perimeter of the joint capsule, nonarticular structures were concealed from the scanning laser beam, thereby restricting blood flow assessment to the articular microvasculature only. At each point in the scan, a blood perfusion measurement is dynamically captured based on the concentration and speed of circulating erythrocytes in the articular microvasculature. A topographical perfusion image of the joint is rapidly generated, colour coded and finally stored for later analysis. The scanner head was positioned 30 cm above the knee and image resolution was set at 93×88 pixels with a scan speed of $4\,ms\,pixel^{-1}$. In order to gain a better appreciation of drug time course, a subset of experiments was undertaken in which the LDI was used in single-point measurement mode to maximise the temporal resolution of the imager so that blood flow measurements can be continuously acquired in real time. Here, the LDI laser beam was directed onto the surface of the joint and blood flow recording commenced at a discrete locus. Test drugs were then topically applied to the joint and perfusion measurements were continuously obtained to determine the time course of the drug. Subsequent scans of the joint could then be arranged in such a way so as to capture the maximal vasomotor response of the agent. At the end of the experiment, the animal was killed by anaesthetic overdose (pentobarbital sodium, 240 mg intracardiac) and a scan of the dead animal knee joint was obtained. This 'biological zero' measurement corresponds to tissue optical noise and was typically 5–10% of the control

scan. The 'biological zero' value was subtracted from all image perfusion values prior to data manipulation.

Experimental protocol

All test agents were topically applied to exposed capsular blood vessels as a 0.1 ml bolus in such a way that the drug was constrained to the knee joint. Following topical application of ACEA (10^{-14}–10^{-9} mol), LDI scans of the knee joint were taken before (control) and then at 0, 1, 2, and 5 min following drug administration. A cumulative dose–response curve to ACEA was generated in each animal. Antagonist experiments were performed in separate animal groups and involved assessing ACEA dose responses in the presence of a specific blocker. The antagonist was topically applied to the joint a few seconds prior to the administration of each dose of ACEA to avoid residual antagonist accumulation on the joint surface. The antagonists used in this study were the CB_1 receptor antagonists AM251 (*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) (10^{-9} mol) and AM281 (1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-4-morpholinyl-1*H*-pyrazole-3-carboxamide) (10^{-8} mol), as well as the selective CB_2 receptor antagonist AM630 (6-iodo-2-methyl-1-[2(-4morpholinyl)ethyl]-[1*H*-indol-3-yl](4-methoxyphenyl)methanone) (10^{-8} mol). Doses of antagonists were based on previous successful antagonism in the rat cardiovascular system (Ford *et al*., 2002).

The role of capsaicin-binding $TRPV_1$ receptors in ACEA-mediated vasoregulation was also examined in this study. Dose–response curves to ACEA were repeated in the presence of the $TRPV_1$ receptor antagonist capsazepine (10^{-6} mol), which was administered to the joint immediately prior to each dose of ACEA. A further group of animals underwent selective denervation of unmyelinated joint afferents by capsaicin pretreatment. Following deep anaesthesia with diazepam (2.5 mg kg^{-1} i.p.) and Hypnorm (fentanyl citrate; 0.3 ml kg^{-1} i.m.), 0.2 ml of 1% capsaicin (vehicle: 5% cremophor, 5% ethanol) was injected into the right knee joint of the rat (0.1 ml in the posterior region and 0.1 in the anterior region of the joint). Animals were allowed to recover for 1 week prior to ACEA testing, which has been shown to be of sufficient duration to allow almost complete destruction of articular unmyelinated nerve fibres (Ferrell *et al*., 1992).

Data analysis and statistics

Each perfusion image was analysed with custom LDI processing software (Moor Instruments Ltd, Axminster, U.K.) and the average blood flow to the anteromedial aspect of the joint calculated and expressed in arbitrary perfusion units (PU). Blood flow changes in response to drug activity were calculated as the percent change in PU between control and test images. All data conformed to a Gaussian distribution and were therefore tested by parametric one- or two-way ANOVA using GraphPad Prism software (GraphPad Software, San Diego, CA, U.S.A.). Paired Student *t*-tests were used to determine any effect of the CB antagonists on joint basal blood flow. A *P*-value <0.05 was considered statistically significant and all data points were expressed as mean $\pm$ s.e.m. for *n* observations.

Drugs

AM251, AM281, AM630, and capsazepine were all supplied by Tocris Cookson Inc. (Ellisville, MO, U.S.A.). ACEA, capsaicin, and urethane were obtained through Sigma-Aldrich Ltd (Ontario, Canada). Hypnorm was purchased from Janssen Pharmaceutica (Beerse, Belgium) and diazepam from Sabex Inc. (Boucherville, Canada). ACEA and all CB antagonists were dissolved in dimethylsulphoxide (DMSO) and cremophor before diluting to the working doses, alliquoting, and storing at either 4°C or −20°C as required. The final concentration of vehicle was maintained at 2% DMSO and 1% cremophor for each dose and this vehicle was found to have no significant effect on joint blood flow (see Figure 1a).

Results

Effects of ACEA and CB antagonists

Topical application of ACEA to the exposed rat knee joint caused a transient increase in perfusion indicative of synovial vasodilatation. Single-point measurement analysis of blood flow revealed that perfusion rose quickly in response to ACEA

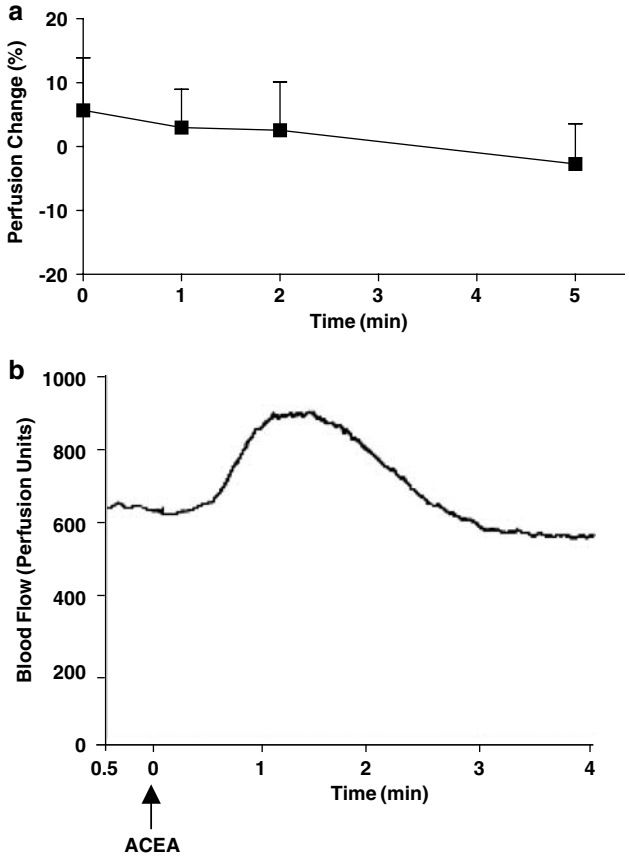

Figure 1 (a) Time course of vehicle (2% DMSO/1% cremophor) on knee joint blood flow. Vehicle was found to have no significant effect on synovial perfusion over the 5 min test period ($P=0.86$; one-way ANOVA, $n=11$). Data are means $\pm$ s.e.m. (b) Representative single-point measure of knee joint perfusion showing real-time dilator effect of topically applied ACEA (10^{-9} mol). Maximal increase in blood flow occurred about 1 min after drug application and then rapidly returned to control levels by about 3 min.

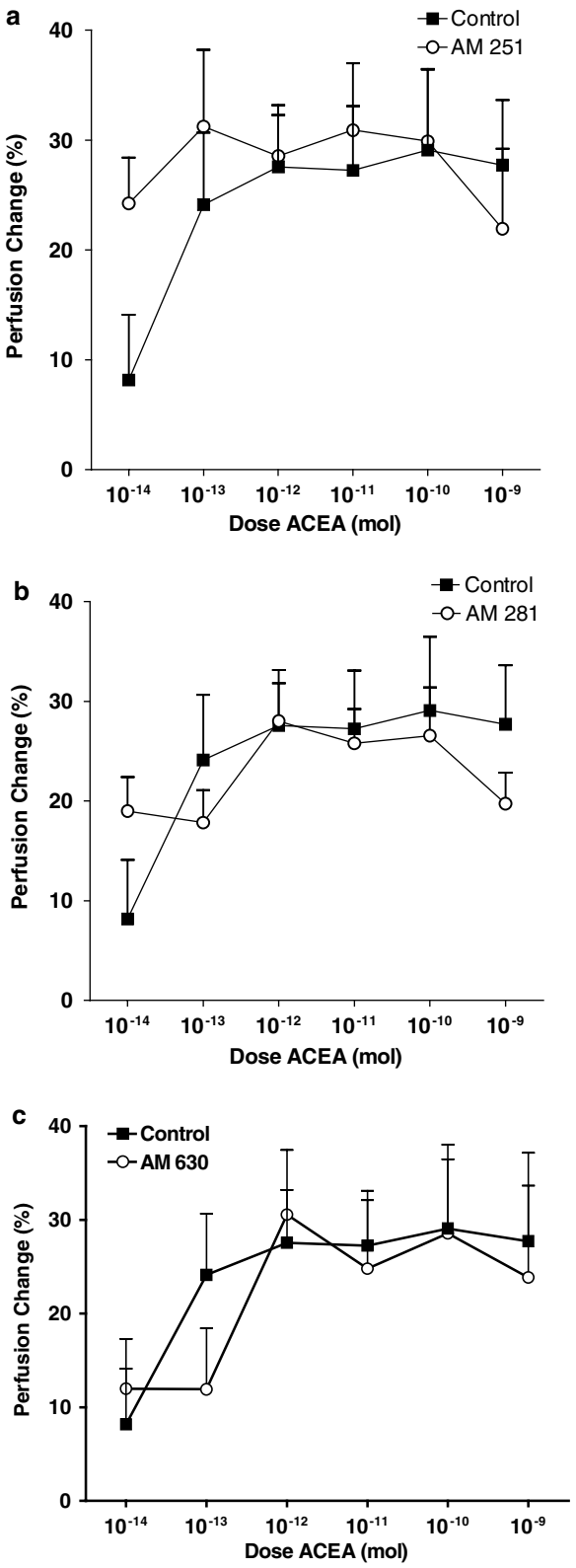

Figure 2 Cumulative dose–response curves to ACEA either alone or in the presence of the CB_1 receptor antagonists AM251 (a) (10^{-9} mol) or AM281 (b) (10^{-8} mol), or the selective CB_2 receptor antagonist AM630 (c) (10^{-8} mol). In each case, the vasodilator effect of ACEA was not blocked by any of the CB receptor antagonists tested ($P=0.29$, 0.52, and 0.63 for AM251, AM281, and AM630, respectively; two-way ANOVA; $n=9$–12). The same control data is shown for each graph. Means ± s.e.m. are indicated.

with maximal vasodilatation occurring approximately 1 min after drug administration (Figure 1b). Joint perfusion subsequently showed a rapid recovery such that blood flow returned to control levels at about the 3 min time point. When administered across the dose range 10^{-14}–10^{-9} mol, ACEA caused a dose-dependent increase in synovial blood flow ($P=0.02$, repeated measures one-way ANOVA; $n=11$) with a maximal perfusion increase of about 30% and an ED50 of $2.20\pm0.01\times10^{-14}$ mol (Figure 2). Coadministration of ACEA with either 10^{-9} mol AM251 (Figure 2a) or 10^{-8} mol AM281 (Figure 2b) showed that neither of these CB_1 receptor antagonists had any effect on the dilator response to ACEA. To test any potential involvement of CB_2 receptors in ACEA-mediated vasodilatation, the specific CB_2 receptor antagonist AM630 (10^{-8} mol) was assessed (Figure 2c) and found to have no significant effect on ACEA-induced hyperaemia.

When each of the CB receptor antagonists alone were independently applied to exposed joints, they were found to have no discernible effect on basal joint perfusion (Figure 3). Thus, the CB antagonists themselves were not acting as partial or inverse agonists to influence joint blood flow in this preparation.

Assessment of $TRPV_1$ receptor involvement in ACEA-dependent vasoactivity

Blockade of the vanilloid receptor $TRPV_1$ with capsazepine attenuated the dilator effect of topically applied ACEA (Figure 4a). This antagonistic effect of capsazepine was consistent across the entire dose range of ACEA. Further evidence of $TRPV_1$ receptor involvement came from experiments in which ACEA failed to alter synovial blood flow in animals whose capsaicin-sensitive nerves had been chemically destroyed (Figure 4b). Thus, the dose–response relationship with ACEA was completely abolished in capsaicin-treated knees.

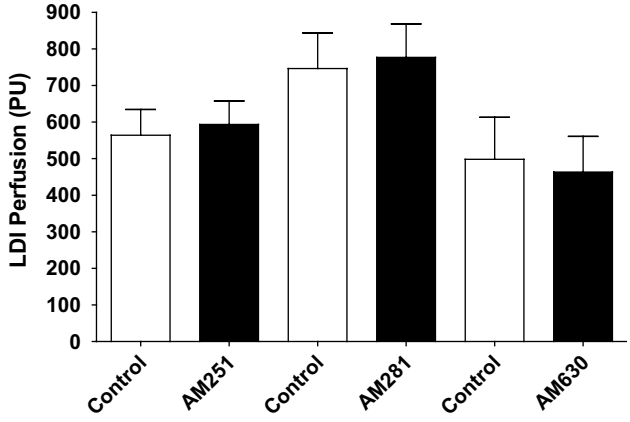

Figure 3 Effect of the selective CB_1 receptor antagonists AM251 (10^{-9} mol) and AM281 (10^{-8} mol), as well as the CB_2 receptor antagonist AM630 (10^{-8} mol) on knee joint basal perfusion. Synovial blood flow was unaltered compared to control following topical administration of any of these CB antagonists ($P=0.10$, 0.15, and 0.39 for AM251, AM281, and AM630 respectively; paired Student t-test; $n=8$–9). Data are shown as means ± s.e.m.

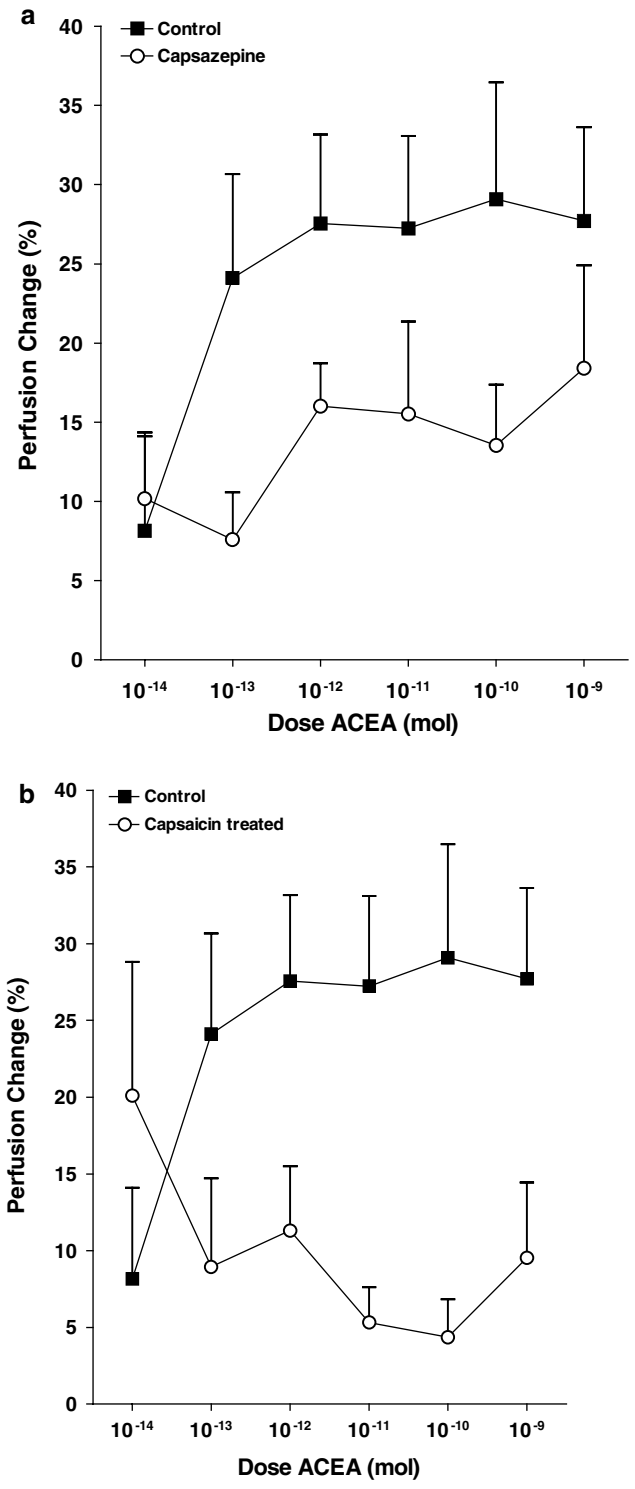

Figure 4 Response to topical application of ACEA on synovial perfusion and the inhibition of this vasodilatation by 10^{-6} mol of the $TRPV_1$ receptor antagonist capsazepine (a). Destruction of unmyelinated joint afferent nerve fibres by capsaicin pretreatment also significantly attenuated the dilator effect of ACEA (b) $P=0.002$ and $P<0.0005$ for the capsazepine- and capsaicin-treated joints, respectively; two-way ANOVA; $n=6$–11. Means±s.e.m. are shown for all data.

Table 1 MAP taken at 1 min following topical ACEA, cannabinoid antagonists, and capsazepine administration

Drug	*MAP* (mmHg)	N-*values*
ACEA (mol)		
Control	76±4	8
10^{-14}	76±3	8
10^{-13}	76±4	8
10^{-12}	75±4	8
10^{-11}	74±4	7
10^{-10}	75±4	7
10^{-9}	68±7	7
AM 251 (mol)		
Control	73±9	9
10^{-9}	74±9	9
AM 281 (mol)		
Control	76±4	12
10^{-8}	71±4	12
AM 630 (mol)		
Control	76±7	8
10^{-8}	74±7	8
Capsazepine (mol)		
Control	60±2	10
10^{-6}	59±2	10

Values are expressed as means±s.e.m.

Blood pressure

Blood pressure was monitored for the duration of all experiments and, as shown in Table 1, none of the drugs investigated had any observable effect on MAP. Thus, the blood flow changes described are local vasomotor responses and not due to systemic uptake of the test agents, leading to alterations in blood pressure and consequently joint perfusion.

Discussion

In addition to their psychotropic and analgesic effects, a growing body of evidence indicates that CBs can also exert potent but transient cardiovascular effects such as bradycardia, hypotension and vasodilatation. This study found that ACEA, a synthetic analogue of the endocannabinoid anandamide, caused a marked increase in synovial blood flow, which was rapid in onset (<1 min) and transient. This hyperaemic response to ACEA was localised to the joint and cannot be attributable to a systemic effect of the CB acting on multiple vascular beds, since mean arterial blood pressure was unaffected by topical administration of the drug at the specified doses (see Table 1). Although ACEA is a highly selective agonist for the CB_1 receptor with a low affinity for CB_2 receptors (Hillard *et al.*, 1999), the dilator effect of the drug in the rat knee was not blocked by any of the CB antagonists tested. Thus, the dilator effect of ACEA was unaltered following coadministration of the CB with the CB_1 receptor antagonists AM251 and AM281, as well as in the presence of the CB_2 receptor antagonist AM630. This finding is consistent with previous studies that have clearly shown that anandamide responses in certain vascular beds are not mediated by known CB receptors (Zygmunt *et al.*, 1999;

Ralevic *et al*., 2000; Smith & McQueen, 2001; Luk & Lam, 2004). These studies went on to show that the vasomotor effects of anandamide in these tissues were, in fact, due to the activation of the capsaicin-preferring, heat-sensitive vanilloid receptor $TRPV_1$. The present investigation supports this mode of action since the selective $TRPV_1$ receptor antagonist capsazepine completely blocked the vasodilatatory effect of ACEA on synovial blood vessels. Furthermore, destruction of unmyelinated and thinly myelinated neurones by capsaicin pretreatment abolished the dilator response to ACEA in these joints, indicating that the site of action of the CB is likely *via* $TRPV_1$ receptors located on capsaicin-sensitive joint afferents, although the evidence presented here is somewhat indirect. Elsewhere, nerve recordings from rat knee joint afferents revealed that anandamide sensitised nociceptors present on capsaicin-sensitive nerves and that this effect was blocked by capsazepine (Gauldie *et al*., 2001). The data presented here corroborate this finding by showing a vascular consequence to joint afferent $TRPV_1$ receptor activation by a CB and extend this observation by establishing that neither CB_1 nor CB_2 receptors are involved in mediating this response. Other experiments performed on isolated rat mesenteric arteries suggests that anandamide binds to $TRPV_1$ receptors located on perivascular C and Aδ fibres to cause the secondary release of inflammatory neuropeptides which in turn generate vascular smooth muscle relaxation (Zygmunt *et al*., 1999; Ralevic *et al*., 2000). Since dilator neuropeptides such as substance P and calcitonin gene-related peptide are known to be vasoactive in joints (McDougall *et al*., 1994; Ferrell *et al*., 1997), then the CB-induced hyperaemia outlined here could conceivably be ascribed to the activation of articular sensory nerves leading to the peripheral release of inflammatory neuropeptides.

An alternative explanation for the hypotensive and dilator effects of CBs has been proposed in which sympathetic neurotransmission is inhibited such that noradrenergic vasoconstrictor tone is attenuated (Varga *et al*., 1995; 1996). Evidence was presented wherein anandamide caused a CB_1-dependent prejunctional inhibition of noradrenaline release from sympathetic nerve terminals. This potential mechanism is unlikely to be occurring in the joint since intra-articular capsaicin injection does not affect sympathetic vasomotor control of articular blood vessels (Karimian *et al*., 1995), whereas the ACEA effects described here were abolished following capsaicin treatment. Both synthetic CBs and endocannabinoids have been shown elsewhere to have no influence on noradrenaline release or on the constrictor response to electrical stimulation of sympathetic perivascular nerves (Malinowska *et al*., 1997; Lay *et al*., 2000). Moreover, the haemodynamic effects of the CB_1 receptor agonist HU210 are sustained in animals that have undergone either supramaximal sympathetic blockade or chemical sympathectomy (Vidrio *et al*., 1996; Wagner *et al*., 2001).

To date, it is unclear if endocannabinoids are vasoactive under normal physiological conditions or whether tissue injury is a prerequisite to induce endocannabinoid activity. In the present study, local administration of CB_1 and CB_2 receptor agonists were themselves found to have no discernible effect on synovial blood flow, indicating that endocannabinoids are not released tonically in the normal uninflamed knee joint to regulate basal joint perfusion. Whether endocannabinoids show a similar vasoactive profile in models of joint injury and arthritis is unknown and should be considered.

Endocannabinoid signalling is rapidly terminated in central and peripheral tissues by a highly efficient transport mechanism such that the ligand is taken up by cells and can therefore no longer access extracellular receptor-binding sites (Di Marzo *et al*., 1994; Beltramo *et al*., 1997; Fowler & Jacobsson, 2002). Once translocated intracellularly, CBs are enzymatically destroyed by the hydrolytic activity of fatty acid amide hydrolase (Deutsch & Chin, 1993; Di Marzo *et al*., 1994). Since this transport process is known to occur in the mammalian cardiovascular system (Calignano *et al*., 1997), then this mechanism would explain the short-lived vasomotor response to ACEA found in the present investigation. Nevertheless, future studies examining the existence and function of a CB transport mechanism in joints needs to be explored.

In conclusion, the synthetic anandamide analogue ACEA causes vasodilatation of rat synovial blood vessels when applied topically to the knee joint. Rather than acting on classic CB receptors, ACEA appears to exert its dilator effect *via* $TRPV_1$ receptors located on capsaicin-sensitive unmyelinated and thinly myelinated joint afferents. These findings suggest that as in other peripheral tissues, endocannabinoids are likely endogenous candidate ligands for synovial $TRPV_1$ receptors and may contribute to the development and progression of joint neurogenic inflammation.

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