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Heterogeneity in the mechanisms of vasorelaxation to anandamide in resistance and conduit rat mesenteric arteries

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1 In order to address mechanistic differences between arterial vessel types, we have compared the vasorelaxant actions of anandamide in resistance (G3) and conduit (G0) mesenteric arteries.

2 Anandamide produced concentration-dependent relaxations of pre-constricted G3 arteries with a maximal response that was significantly greater than seen in G0.

3 The CB_1 receptor selective antagonists SR141716A (100 nM) and AM251 (100 nM) caused reductions in the vasorelaxant responses to anandamide in both arteries. Maximal vasorelaxant responses to anandamide were reduced in both arteries after treatment with capsaicin to deplete sensory neurotransmitters (10 μM for 1 h).

4 Vasorelaxation to anandamide was not affected by the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 300 μM) in either artery. Only responses in G3 arteries were sensitive to removal of the endothelium. In G3 vessels only, vasorelaxation to anandamide was reduced by inhibition of EDHF activity with a combination of charybdotoxin (100 nM) and apamin (500 nM) in the presence of L-NAME (300 μM) and indomethacin (10 μM).

5 Antagonism of the novel endothelial cannabinoid receptor (O-1918, 1 μM) caused a reduction in the sensitivity to anandamide in G3 but not G0.

6 G3, but not G0, vessels showed a small reduction in vasorelaxant responses to anandamide after inhibition of gap junctional communication with 18α-GA (100 μM).

7 These results demonstrate that there are differences in the mechanisms of vasorelaxation to anandamide between conduit and resistance mesenteric arteries. In small resistance vessels, vasorelaxation occurs through stimulation of vanilloid receptors, CB_1 receptors, and an endothelial receptor coupled to EDHF release. By contrast, in the larger mesenteric artery, vasorelaxation is almost entirely due to stimulation of vanilloid receptors and CB_1 receptors, and is endothelium-independent.

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Keywords: Mesenteric artery (rat); cannabinoids; anandamide; vanilloid; vasorelaxation; endothelium; EDHF; gap junctions

Abbreviations: 18α-GA, 18α-glycyrrhetinic acid; AM251, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide; CB, cannabinoid; ChTX, charybdotoxin; EDHF, endothelium-derived hyperpolarizing factor; G0, the superior mesenteric artery; G3, third-order branch of the superior mesenteric artery; L-NAME, N^G-nitro-L-arginine methyl ester; SR141716A, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide

Introduction

Anandamide has been shown to cause vasorelaxation in a number of tissues, and while several putative mechanisms have been put forward to explain this phenonomen, there are still substantial discrepancies in the literature. To date, mechanisms implicated include nitric oxide release, metabolism to vasoactive arachidonic metabolites, prostanoid involvement, endothelium-derived hyperpolarising factor (EDHF) release, and inhibition of calcium channels (for a review see Randall *et al*., 2002). Additionally, Zygmunt *et al*. (1999) showed that some of the effects of anandamide in the rat arteries are mediated through the stimulation of vanilloid receptors on sensory nerves, with the subsequent release of the vasodilator neurotransmitter calcitonin gene-related peptide (CGRP). While this has been widely confirmed in the rat mesentery (Ralevic *et al*., 2000; Harris *et al*., 2002), in coronary vessels from several species it has been consistently shown that there is no involvement of vanilloid receptors (Grainger & Boachie-Ansah, 2001; White *et al*., 2001; Ford *et al*., 2002). This might suggest that the actions of anandamide may be dependent on vanilloid receptor density, and/or perivascular nerve density in a given blood vessel. Indeed, Andersson *et al*. (2002) showed in guinea-pigs that, while anandamide is a full agonist at the vanilloid receptor in mesenteric arteries, it is a weak agonist of this receptor in main bronchi. Vanheel & Van De Voorde (2001) also reported that anandamide produces capsazepine-sensitive hyperpolarisations of daughter branches of the mesenteric artery, but not of the superior mesenteric artery. Such differences in vanilloid receptor expression may explain variations in vasorelaxant responses to anandamide between vascular beds, or indeed between vessels within the same bed.

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Whether vasorelaxant responses to anandamide are endothelium-dependent is unclear from the literature (Chaytor *et al*., 1999; Wagner *et al*., 1999; White *et al*., 2001; Ho & Hiley, 2003). However, it is clear that some cannabinoids such as abnormal cannabidiol act in an endothelium-dependent manner (Jarai *et al*., 1999; Ho & Hiley, 2003) and it is proposed that an endothelial cannabinoid receptor exists (Jarai *et al*., 1999; Offertaler *et al*., 2003). We have recently demonstrated that the vasodilator response to a novel endocannabinoid, *N*-arachidonoyl-dopamine (NADA), is endothelium-dependent in daughter vessels of the superior mesenteric artery only (O'Sullivan *et al*., 2004). This might suggest that there is differential expression of the novel endothelial receptor between arteries.

The involvement of currently recognised cannabinoid (CB) receptors in vasorelaxation to anandamide is unclear. While there is evidence that CB_1 receptors exist on smooth muscle (Darker *et al*., 1998), it is unclear whether these are coupled to vasorelaxation. Previous studies have implicated CB_1 receptors in vasorelaxation to anandamide on the basis of inhibition by SR141716A, a CB_1 receptor antagonist. However, other CB_1 receptor antagonists such as AM251 or LY320135 have not been successful in inhibiting vasorelaxation to anandamide (White *et al*., 2001; Harris *et al*., 2002). Furthermore, the use of SR141716A at higher concentrations is complicated in that SR141716A can inhibit myoendothelial gap junctions (Chaytor *et al*., 1999) and may have actions at the vanilloid receptor (De Petrocellis *et al*., 2001; Millns *et al*., unpublished data).

In order to address the issue of differential responses to anandamide between arterial vessel types, we have investigated the vasorelaxant actions of anandamide in resistance compared with conduit mesenteric vessels. Specifically, the role of various receptors, the endothelium and the mechanisms of vasodilatation has been investigated. The role of the novel endothelial receptor was examined using a new antagonist, O-1918 (Offertaler *et al*., 2003).

Methods

Male Wistar rats (250–350 g) were stunned by a blow to the back of the head and killed by cervical dislocation. The superior mesenteric artery and mesenteric arterial bed were removed rapidly and placed into cold Krebs–Henseleit buffer (composition, mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2, D-glucose 10). From the mesenteric arterial bed, segments of 2 mm third-order branches of the superior mesenteric artery (G3) were dissected free of adherent connective and adipose tissue. G3 vessels (200–400 μm diameter) were mounted on fine tungsten wires (40 μm diameter) on a Mulvany–Halpern myograph (Myo-Interface Model 410A, Danish Myo Technology, Denmark) (Mulvany & Halpern, 1977). The superior mesenteric artery (G0; 3–4 mm in length, 1–1.5 mm diameter) was also cleaned of adherent tissue and was mounted on fixed segment support pins using a Multi Myograph system (Model 610 M, Danish Myo Technology, Denmark). Tension was measured and was recorded on a MacLab 4e recording system (ADInstruments, U.K.).

Once mounted, all vessels were kept at 37°C in Krebs–Henseleit buffer and gassed with 5% CO_2 in O_2. The mesenteric vessels were stretched to an optimal passive tension of 5 mN and allowed to equilibrate. The contractile integrity of each vessel was then tested by its ability to contract to 60 mM KCl by at least 5 mN.

In some preparations of both vessel types, the endothelium was removed by abrasion with a human hair (White & Hiley, 1997). Preparations were considered to be denuded when relaxation to 10 μM carbachol after pre-constriction with U46619 was less than 20% relaxation of the imposed contraction (see White & Hiley, 1997). All other G3 and G0 preparations were endothelium-intact.

Experimental protocol

Viable vessels were contracted with U46619 (10–300 nM), a thromboxane mimetic, to increase tension by at least 5 mN. The average increase in tension for G3 vessels was 10.2 ± 0.4 mN ($n = 70$), and for the superior mesenteric artery was 18.8 ± 0.6 mN ($n = 65$). Once a stable contraction was achieved and maintained, the vasorelaxant effects of anandamide were assessed as cumulative concentration–response curves by the addition of stock solutions to the buffer. The steady-state response to anandamide was taken at each concentration and expressed as a percentage relaxation of the imposed U46619 contraction. All protocols were carried out in both the superior mesenteric artery (G0) and third-order branches of the superior mesenteric artery (G3) to establish regional differences between resistance and conduit vessels.

The involvement of cannabinoid receptors was assessed using the CB_1 receptor antagonists SR141716A and AM251, each at 100 nM (Rinaldi-Carmona *et al*., 1996; White *et al*., 2001), and O-1918, an antagonist of the novel endothelial receptor (1 μM, Offertaler *et al*., 2003). These antagonists were added to the preparations 10 min before pre-constriction. In some paired experiments, SR141716A at 100 nM was combined with endothelial denudation and compared with the effects of denudation alone. The vasorelaxant effects of the CB_1 receptor agonist CP55,940 were tested in the presence and absence of SR141716A at 100 nM. To assess the involvement of vanilloid receptors, some vessels were incubated for 1 h with the vanilloid agonist capsaicin (10 μM) to deplete the sensory nerves of neurotransmitters, followed by a 20 min washout period (Zygmunt *et al*., 1999; Harris *et al*., 2002).

The involvement of nitric oxide was investigated by inhibiting nitric oxide synthase with N^G-nitro-L-arginine methyl ester (L-NAME, 300 μM, Randall & Griffith, 1991). In these experiments, L-NAME was present in the buffer throughout the whole experiment. To inhibit EDHF activity, endothelium-intact preparations were incubated with charybdotoxin, to inhibit large-conductance calcium-activated K^+ channels and voltage-sensitive K^+ channels (ChTX, 100 nM), and apamin, to inhibit small conductance calcium activated K^+ channels (500 nM) in the presence of L-NAME (300 μM) and indomethacin (10 μM) to inhibit nitric oxide and prostanoid synthesis, respectively (McCulloch *et al*., 1997). In the presence of L-NAME and indomethacin, the combination of ChTX and apamin inhibits EDHF responses (Zygmunt *et al*., 1997; Randall & Kendall, 1998). In some experiments in G3 vessels, indomethacin (10 μM) alone was present in the buffer to inhibit prostanoid synthesis. The gap junction inhibitor 18α-glycyrrhetinic acid (18α-GA, 100 μM; Chaytor *et al*., 1999) was used to test whether actions on the endothelium are communicated to the smooth muscle *via* gap junctions.

To investigate whether O-1918 reduced EDHF-mediated responses nonspecifically, vessels were pre-incubated with O-1918 (1 μM) and the vasorelaxation to 10 μM carbachol was tested against control vessels.

Statistical analysis

The concentration of vasorelaxant giving a half-maximal response (EC_{50}) was obtained from the concentration–response curve fitted to a sigmoidal logistic equation using the GraphPad Prism package (Tep-areenan *et al*., 2003), and expressed as its negative logarithm, pEC_{50}. Maximal and pEC_{50} responses are expressed as mean$\pm$s.e.m. The number of animals in each group is represented by *n*. Data were compared by analysis of variance (ANOVA) with statistical significance between manipulations and controls determined by Dunnett's *post-hoc* test.

Drugs

All drugs were supplied by Sigma Chemical Co. (Poole, U.K.) except where stated. Carbachol and L-NAME were dissolved in Krebs–Henseleit solution. Anandamide, CP55,940, capsaicin, indomethacin and SR141716A were dissolved in ethanol at 10 mM with further dilutions made in distilled water. Anandamide, AM251 and CP55,940 were obtained from Tocris (U.K.). SR141716A was supplied by Research Biochemicals International as part of the Chemical Synthesis Programme of the National Institute of Mental Health contract (NOIMH3003). AM251 was a kind gift from Professor Sheila Gardiner and was initially dissolved in DMSO to a concentration of 10 mM, with further dilutions in distilled water. 18α-GA was also dissolved in DMSO. O-1918 was generously donated by Dr George Kunos and was dissolved in ethanol at a stock concentration of 200 mM.

Results

Anandamide produced concentration-dependent relaxations of endothelium-intact, U46619 pre-contracted G3 and G0 arteries. Both the potency ($P<0.05$) and the reactivity ($P<0.01$) of anandamide were significantly greater in G3 compared to G0 arteries (Figure 1, Table 1).

Cannabinoid and vanilloid receptor involvement

In G3 arteries, the CB_1 receptor antagonist SR141716A (100 nM) caused a significant rightward shift in the concentration–response curve to anandamide ($P<0.01$, Figure 2a). Similarly, in G0, SR141716A significantly reduced the vasorelaxant responses of anandamide ($P<0.01$, Table 1, Figure 2d). The CB_1 receptor antagonist AM251 (100 nM) significantly reduced the maximal vasorelaxant response to anandamide in G3 ($P<0.05$, Figure 2b) and GO ($P<0.01$, Table 1, Figure 2e). In paired vessels, when SR141716A (100 nM) was added to G3 vessels following endothelial denudation, the vasorelaxant effect of anandamide was reduced further than was seen with denudation alone (denuded vessels, $pEC_{50}=5.12\pm0.11$, $n=7$; denudation in the presence of SR141716A, $pEC_{50}=4.45\pm0.14$, $n=7$, $P<0.01$, Figure 4a). The CB_1 receptor agonist CP55,940 caused vasorelaxation of G3 vessels ($R_{max}=94.5\pm2.6$ % relaxation, $pEC_{50}=5.97\pm0.05$, $n=5$). Addition of SR141716A (100 nM) caused a significant rightward shift of the CP55,940 curve ($pEC_{50}=5.44\pm0.06$, $n=7$, $P<0.01$, Figure 4b).

Pre-treatment with the vanilloid receptor agonist capsaicin (10 μM) significantly reduced the maximal vasorelaxant responses to anandamide in both G3 and G0 vessels ($P<0.01$, Table 1, Figure 2c, f).

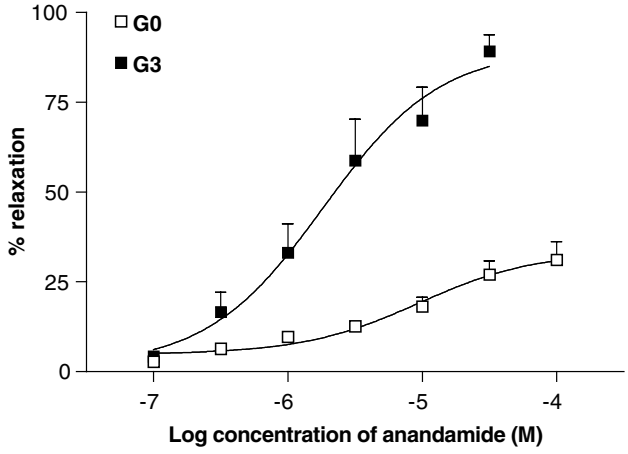

Figure 1 The vasorelaxant effects of anandamide in resistance (G3) mesenteric vessels compared with the superior mesenteric artery (G0). Data are given as means with error bars representing s.e.m.

Table 1 Effects of various treatments on the vasorelaxation responses to anandamide in the rat superior mesenteric artery compared with third-order branches of the superior mesenteric artery

	G3			*G0*		
	pEC_{50}	R_{max} (%)	n *number*	pEC_{50}	R_{max} (%)	n *number*
Control	5.74$\pm$0.20	89.8$\pm$7.6	9	5.02$\pm$0.21	33.4$\pm$3.3	7
SR141716A (100 nM)	4.87$\pm$0.26**	71.5$\pm$14.6*	6	4.10$\pm$0.76**	32.1$\pm$24.8	6
AM251 (100 nM)	6.43$\pm$0.97*	48.9$\pm$10.3**	6	6.01$\pm$0.56	12.9$\pm$1.9**	6
Capsaicin pre-treatment (10 μM)	5.03$\pm$0.35*	51.2$\pm$12.4**	6	4.55$\pm$0.38	11.1$\pm$3.9**	5
O-1918 (1 μM)	4.82$\pm$0.22**	107$\pm$22	6	5.31$\pm$0.38	32.5$\pm$5.5	6
Endothelium denuded	5.08$\pm$0.29	65.2$\pm$13.4**	6	4.82$\pm$0.31	37.6$\pm$6.6	7
L-NAME (300 μM)	5.17$\pm$0.48	79.7$\pm$15.8	6	5.01$\pm$0.37	42.7$\pm$8.2	6
ChTx (100 nM) & apamin (500 nM)	3.44$\pm$0.20**	47.9$\pm$10.2**	6	4.44$\pm$0.27	44.3$\pm$9.2	7
With L-NAME (300 μM) & indomethacin (10 μM)						
18αGA	5.87$\pm$0.30	59.2$\pm$7.8**	6	5.84$\pm$0.30	25.1$\pm$2.04	7

*denotes a significant inhibitory effect of treatment compared with control vasorelaxant responses to anandamide ($P<0.05$, **$P<0.05$).

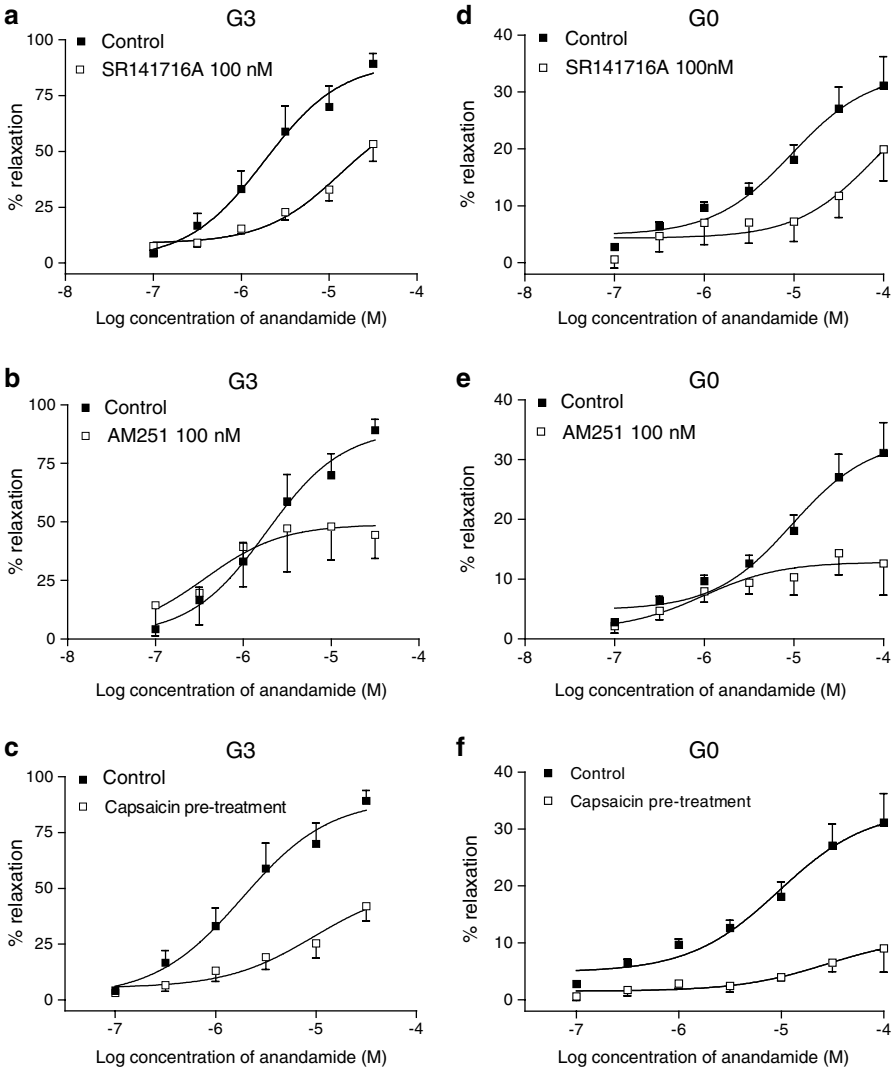

Figure 2 Effects of the CB_1 receptor antagonists SR141617A (100 nM (a, d)) and AM251 (100 nM (b, e)), and pre-treatment with the vanilloid receptor agonist, capsaicin (10 μM (c, f)) on vasorelaxant responses to anandamide in G3 compared with G0. Data are given as means with error bars representing s.e.m.

Endothelial component of anandamide-induced vasorelaxation

Removal of the endothelium resulted in a significant reduction in the vasorelaxant responses to anandamide in G3 vessels ($P<0.01$, Table 1, Figure 3a). By contrast, de-endothelialisation had no effect on responses to anandamide in G0 vessels (Figure 3e).

The presence of L-NAME did not affect vasorelaxation to anandamide in either vessel type tested (Figure 3b and f). The combination of ChTx (100 nM) and apamin (500 nM) in the presence of L-NAME (300 μM) and indomethacin (10 μM) caused a significant reduction in vasorelaxation to anandamide in G3 ($P<0.01$, Figure 3c), but not in G0 vessels (Figure 3g). Indomethacin (10 μM) alone had no effect on the response to anandamide in G3 vessels ($R_{max}=96.3\pm9.2\%$ relaxation, $pEC_{50}=5.50\pm0.19$, $n=6$, Figure 3b). The gap junction inhibitor 18α-GA (100 μM) had a significant inhibitory effect on the maximal vasorelaxant response to anandamide in G3 vessels ($P<0.01$, Figure 3d), but did not affect the vasorelaxation to anandamide in the superior mesenteric artery (Figures 3h and 4).

In G3 arteries, O-1918 (1 μM) caused a significant rightward shift in the concentration–response curve to anandamide without affecting the maximal response ($P<0.01$, Figure 5a). By contrast, O-1918 (1 μM) had no effect on the vasorelaxant responses to anandamide in G0 vessels (Figure 5b). O-1918 (1 μM) did not affect vasorelaxation to the endothelium-dependent relaxant carbachol at 10 μM in endothelium-intact G3 vessels (control, $81.0\pm7.0\%$ relaxation, $n=5$; in the presence of O-1918; $83.3\pm4.8\%$ relaxation, $n=5$, Figure 5c).

Discussion

The aim of the present study was to characterise the mechanisms involved in vasorelaxation to anandamide in the superior mesenteric artery compared with its daughter resistance branches. The results of this study show for the

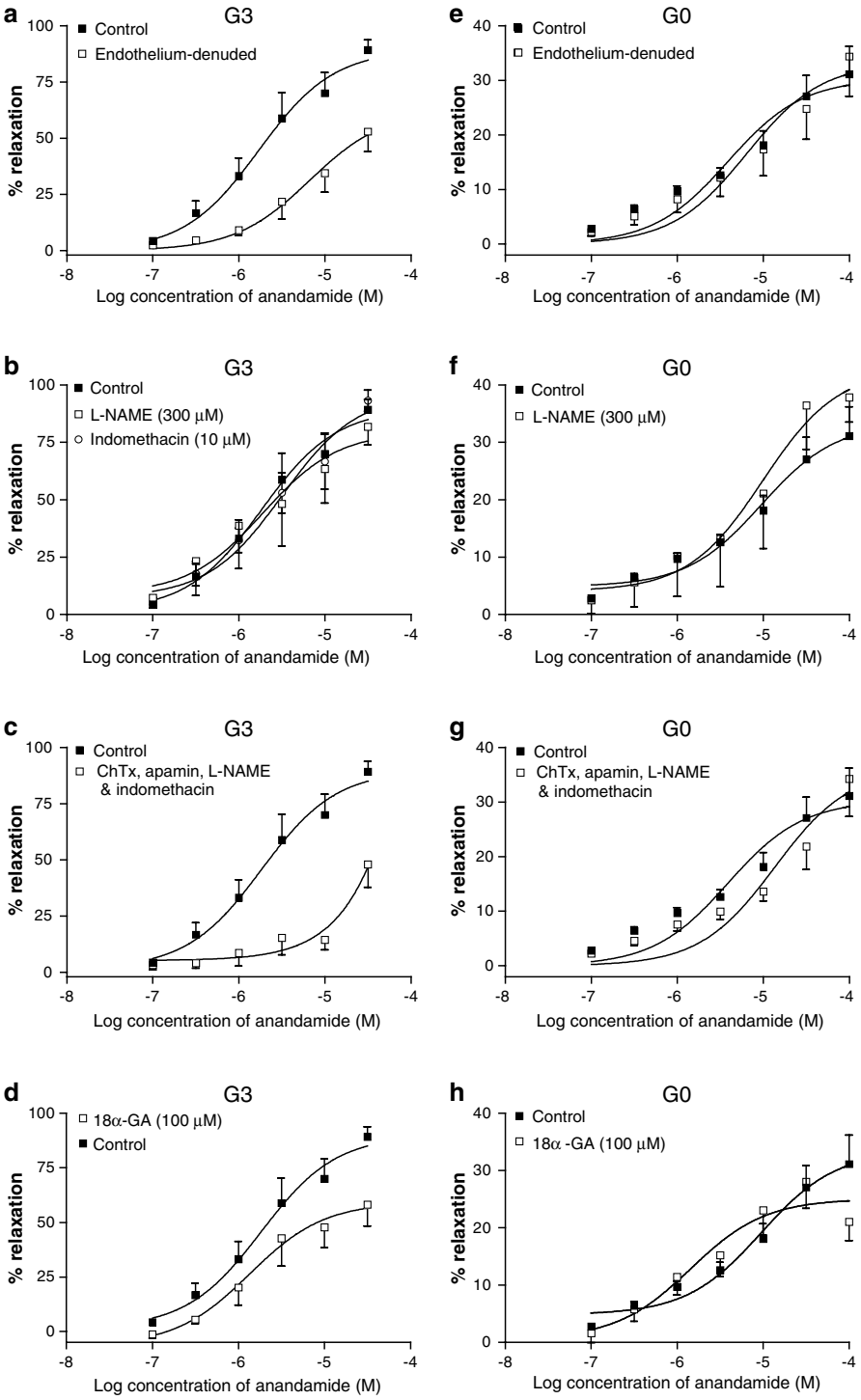

Figure 3 The vasorelaxant responses to anandamide after removal of the endothelium (a, e), after nitric oxide synthase inhibition by L-NAME (300 μM, (b, f)), or in the presence of indomethacin (10 μM, (b)), after inhibition of EDHF activity with ChTX (100 nM) and apamin (500 nM) in the presence of L-NAME (300 μM) and indomethacin (10 μM) (c, g), and after gap junction inhibition with 18α-GA (100 μM, (d, h)) in small resistance vessels (G3) compared with the superior mesenteric artery (G0). Data are given as means with error bars representing s.e.m.

first time that, in small resistance vessels, vasorelaxation occurs through stimulation of the vanilloid receptor, the CB_1 receptor, and an endothelial site of action. Importantly, vasorelaxation to anandamide is partially mediated ($\sim$40%) by EDHF release in G3 vessels only. By contrast, in the larger mesenteric artery, vasorelaxation is due to stimulation of the vanilloid receptor and CB_1 receptor only. These findings support the proposal of a novel endothelial cannabinoid receptor, which is expressed/functional in resistance mesenteric vessels only.

We found that both G3 and G0 vessels are partially mediated by the CB_1 receptor, as indicated by the rightward

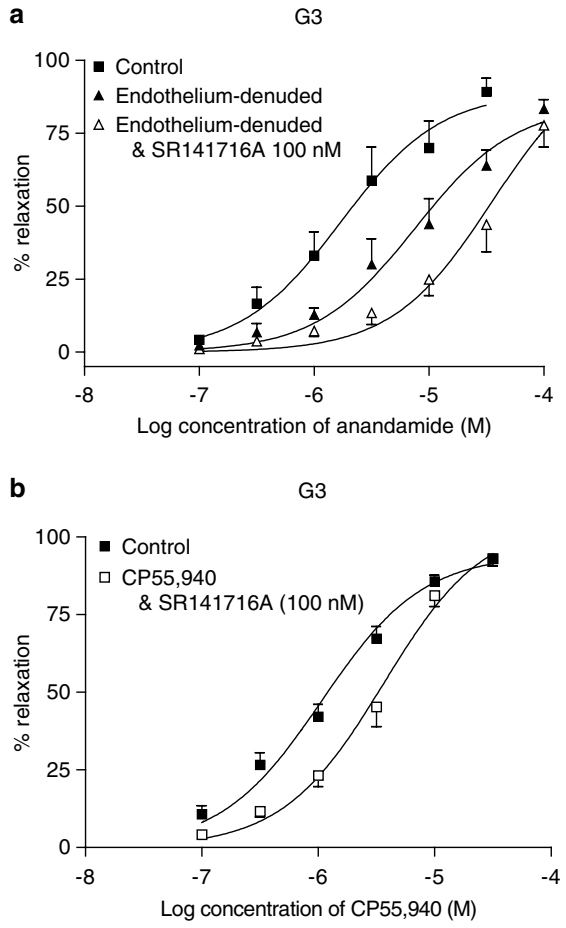

Figure 4 The effects of SR141716A (100 nM) following endothelial denudation on vasorelaxation to anandamide in G3 vessels (a), and the effects of a CB_1 receptor agonist in the presence of SR141716A (100 nM, (b)). Data are given as means with error bars representing s.e.m.

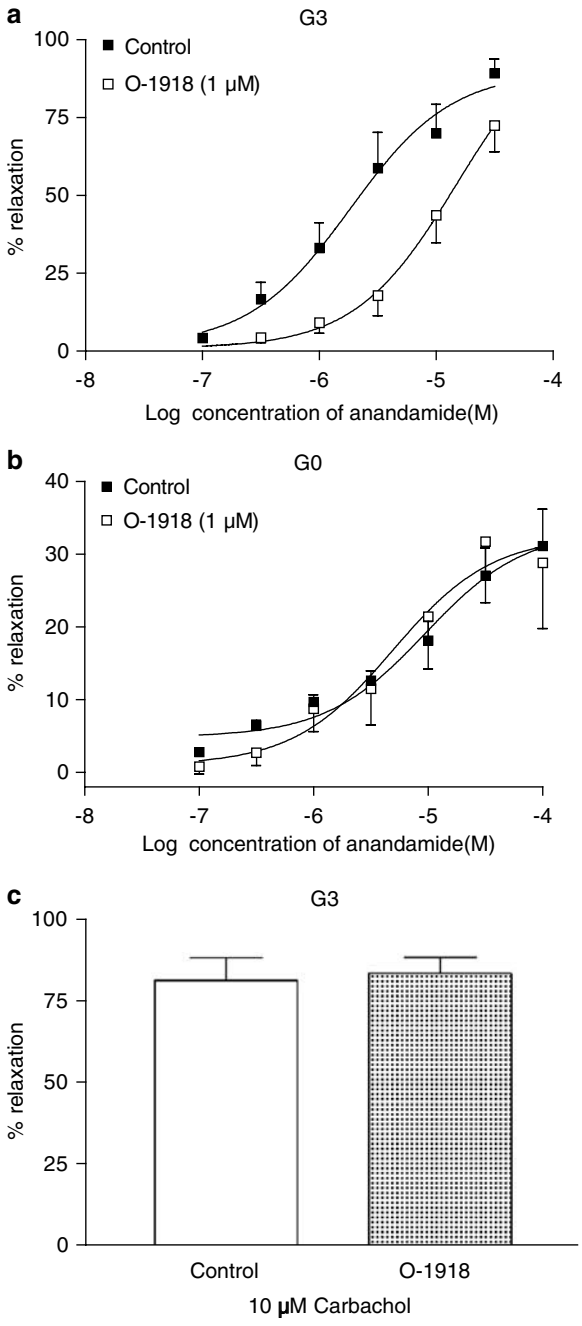

Figure 5 The effects of O-1918 (1 μM) on vasorelaxation to anandamide in G3 and G0, and to carbachol (10 μM) in G3 vessels. Data are given as means with error bars representing s.e.m.

shift in sensitivity to anandamide after CB_1 receptor antagonism with SR141716A at a low concentration (100 nM). Although the shift may appear less than expected on the basis of the kd of SR141716A obtained in rat brains (Rinaldi-Carmona *et al*., 1996), it should be noted that the CB_1 receptor-mediated pathway is not the only mechanism by which anandamide causes vasorelaxation, and so other mechanisms may compensate. Previous studies in isolated mesenteric vessels have shown vasorelaxation to anandamide to be inhibited by higher concentrations of SR141716A (1–10 μM; White & Hiley, 1997; Chaytor *et al*., 1999; Ho & Hiley, 2003), although in these cases, SR141716A may be acting at sites other than the CB_1 receptor. In the present study, SR141716A at 100 nM was still effective at reducing the vasorelaxant effects of anandamide after removal of the endothelium in G3 vessels, showing that the antagonism produced by SR141716A against responses to anandamide is not exclusively through the endothelial receptor, as suggested (Jarai *et al*., 1999; Offertaler *et al*., 2003). Furthermore, the CB_1 receptor agonist CP55,940 also caused relaxation of G3 vessels and this was competitively antagonised by SR141716A. We also found that an alternative CB_1 receptor antagonist, AM251, caused inhibition of vasorelaxation to anandamide in both vessels, although the effects of SR141716A were more consistent with simple competitive antagonism, suggesting that there may be some differences between these structurally similar antagonists.

In 1999, Zygmunt *et al* reported that anandamide caused vasorelaxation through stimulation of vanilloid receptors. Anandamide has since been confirmed as a full agonist at the vanilloid receptor, requiring transport across the cell membrane to activate it (De Petrocellis *et al*., 2001). In the present study, it was found that pre-treatment of either the superior mesenteric artery or small mesenteric vessels with

capsaicin resulted in a reduction in vasorelaxation to anandamide, indicating that sensory nerves play a significant role in mediating vasorelaxation to anandamide in both vessel types. This is in contrast to the work by Andersson *et al*. (2002) who found that vanilloid receptor-mediated hyperpolarisations produced by anandamide were only present in the daughter vessels. In the case of G0, the inhibitory effects of capsaicin point to sensory nerves playing a substantial role, as there was only a 10% residual relaxation after this treatment. However, the presence of the CB_1 receptor antagonist also caused substantial reductions in the responses to anandamide in G0, which might imply that some level of synergy exists between the two vasorelaxant pathways.

The first difference between vessel types appeared following endothelial denudation. In these experiments, removal of the endothelium resulted in approximately a 40% reduction in the vasorelaxant response to anandamide in G3 vessels. In contrast, the responses to anandamide in the superior mesenteric artery were not affected. This is in agreement with our previous findings that another endocannabinoid, *N*–arachidonoyl–dopamine (NADA), caused vasorelaxation in a partially endothelium-dependent manner in G3 but not in G0 (O'Sullivan *et al*., 2004). In the literature, there is conflicting evidence concerning the importance of the endothelium in vasorelaxation to anandamide. Studies have reported a role for the endothelium in vasorelaxation to anandamide in whole perfused mesenteric beds (Wagner *et al*., 1999), and others have not (Harris *et al*., 2002), although it is unclear to what extent various vessels contribute towards the pressure responses observed in the whole bed. In isolated mesenteric preparations, some investigators have also reported endothelium dependence (Chaytor *et al*., 1999) while others have not (White & Hiley, 1997). It must be considered that the importance of the endothelium may depend on the presence or absence of the proposed endothelial cannabinoid receptor in a particular vessel. To test this, we compared the actions of O-1918 in both vessel types. O-1918 acts at the site of vasorelaxant action of abnormal cannabidiol, which has been shown to be neither the CB_1, CB_2 or vanilloid receptor, and is thus proposed to antagonise the novel endothelial receptor (Begg *et al*., 2003; Offertaler *et al*., 2003). In the present study, we found that O-1918 reduced the sensitivity to anandamide in G3 vessels only in a manner typical of competitive antagonism. We have recently published a similar finding for the endocannabinoid NADA (O'Sullivan *et al*., 2004). These data suggest that the proposed novel endothelial cannabinoid receptor may be functional and/or expressed in small resistance arteries only. Since O-1918 had no effect on responses to anandamide in G0, but SR141716A did, this would tend to exclude the possibility that O-1918 is acting at the CB_1 receptor.

To establish the nature of the endothelium-derived vasodilator involved in anandamide-mediated vasorelaxation, we examined the relative contributions of nitric oxide, prostanoids and EDHF activity through inhibition of K^+ channels. We found that nitric oxide was not involved in vasorelaxation to anandamide in either artery tested, which is in accordance with previous findings in this arterial bed (Randall *et al*., 1996; White & Hiley, 1997; Chaytor *et al*., 1999), although some studies report a role for nitric oxide in the kidney (Deutsch *et al*., 1997) or in the aorta (Mukhopadhyay *et al*, 2002). The responses to anandamide in G3 vessels were sensitive to inhibition of EDHF activity by the combination of ChTx and apamin in the presence of L-NAME and indomethacin (Zygmunt *et al*., 1997; Randall & Kendall, 1998). G3 vessels were not sensitive to indomethacin alone, so it is unlikely that anandamide acts by production of vasodilator prostanoids. By contrast, in the superior mesenteric artery, inhibition of EDHF activity did not affect vasorelaxation to anandamide. This may suggest that the endothelial cannabinoid receptor is coupled to EDHF release. Since O-1918 did not inhibit the actions of the endothelium-dependent dilator carbachol, we have also confirmed that O-1918 is not simply inhibiting EDHF activity. Our results clearly show that the endothelial component to vasorelaxation in G3, but not G0, involves EDHF-type relaxations. The difference between G0 and G3 vessels may well be the contribution of EDHF, since, following inhibition of EDHF, the responses in G3 were similar in size and mechanism to those in G0. Indeed it is well established, in terms of endothelial control, that EDHF plays a more prominent role in smaller vessels.

Chaytor *et al*. (1999) reported that anandamide partly acted through the release of EDHF, diffusing to underlying smooth muscle *via* gap junctions. To test this hypothesis, the gap junction inhibitor 18α-GA was tested in G3 and G0 vessels. In agreement with the previous findings, 18α-GA caused a small reduction in the response to anandamide in G3 but not G0, consistent with our proposal that the endothelial pathway involved in vasorelaxation to anandamide is only present in the resistance arteries.

In summary, we have shown clear mechanistic differences in the vasorelaxant effects of the endocannabinoid anandamide between conduit and resistance arteries. Both arteries show relaxation as a consequence of stimulation of the vanilloid receptor and the CB_1 receptor, however, in G3, EDHF activity contributes a further 40–50% to the relaxation response.

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