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REVIEW

Vascular targets for cannabinoids: animal and human studies

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Application of cannabinoids and endocannabinoids to perfused vascular beds or individual isolated arteries results in changes in vascular resistance. In most cases, the result is vasorelaxation, although vasoconstrictor responses are also observed. Cannabinoids also modulate the actions of vasoactive compounds including acetylcholine, methoxamine, angiotensin II and U46619 (thromboxane mimetic). Numerous mechanisms of action have been proposed including receptor activation, potassium channel activation, calcium channel inhibition and the production of vasoactive mediators such as calcitonin gene-related peptide, prostanoids, NO, endothelial-derived hyperpolarizing factor and hydrogen peroxide. The purpose of this review is to examine the evidence for the range of receptors now known to be activated by cannabinoids. Direct activation by cannabinoids of CB_1, CB_e, TRPV1 (and potentially other TRP channels) and PPARs in the vasculature has been observed. A potential role for CB_2, GPR55 and 5-HT_{1A} has also been identified in some studies. Indirectly, activation of prostanoid receptors (TP, IP, EP_1 and EP_4) and the CGRP receptor is involved in the vascular responses to cannabinoids. The majority of this evidence has been obtained through animal research, but recent work has confirmed some of these targets in human arteries. Vascular responses to cannabinoids are enhanced in hypertension and cirrhosis, but are reduced in obesity and diabetes, both due to changes in the target sites of action. Much further work is required to establish the extent of vascular actions of cannabinoids and the application of this research in physiological and pathophysiological situations.

LINKED ARTICLES
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Abbreviations
2-AG, 2-arachidonoylglycerol; Abn-CBD, abnormal cannabidiol; AEA, anandamide; ARA-S, N-arachidonoyl-L-serine; BK_{Ca}, calcium-activated large conductance potassium channel; CB_1, cannabinoid receptor 1; CB_2, cannabinoid receptor 2; CB_e, putative endothelial cannabinoid receptor; CGRP, calcitonin gene-related peptide; GPR119, orphan GPCR; GPR55, orphan GPCR; HBEC, human brain endothelial cell; ICAM, intercellular cell adhesion molecule; LPI, lysophosphatidylinositol; NADA, N-arachidonoyl dopamine; OEA, oleoylethanolamide; PEA, palmitoylethanolamine; PTX, pertussis toxin; THC, Δ^9-tetrahydrocannabinol; TRP, transient receptor potential; TRPV1, transient receptor potential vanilloid 1; VCAM, vascular cell adhesion molecule

Introduction

For many years, it was thought that cannabinoids (chemical constituents of *Cannabis sativa* or structurally related chemicals that bind to cannabinoid receptors) caused their effects through non-specific membrane interactions (Lawrence and Gill, 1975). However, in 1990, the first GPCR with cannabinoid specificity was discovered, called cannabinoid receptor one (CB_1) (Matsuda *et al.*, 1990; Alexander *et al.*, 2013a). A second cannabinoid receptor, CB_2, was cloned in 1993 (Munro *et al.*, 1993). CB_1 and CB_2 are widely distributed, with expression observed in vascular smooth muscle and

endothelial cells (Sugiura *et al.*, 1998; Liu *et al.*, 2000; Rajesh *et al.*, 2007; 2008a). Cannabinoids, endocannabinoids (endogenous cannabinoids) and related endocannabinoid-like compounds also interact with other receptors including an uncloned GPCR located on the endothelium (CB_e, Jarai *et al.*, 1999), the orphan receptor GPR55 (Ryberg *et al.*, 2007; Lauckner *et al.*, 2008), the orphan receptor GPR119 (Overton *et al.*, 2006), transient receptor potential (TRP) channels (Zygmunt *et al.*, 1999; Jordt *et al.*, 2004; De Petrocellis *et al.*, 2007; Qin *et al.*, 2008; Alexander *et al.*, 2013b), PPARα,β,γ (reviewed in O'Sullivan, 2007; Alexander *et al.*, 2013c), opioid receptors (Seely *et al.*, 2012; Zador *et al.*, 2012), adrenoceptors (Cascio *et al.*, 2010) and 5-HT receptors (Russo *et al.*, 2005). However, the pharmacological profiling of these compounds is complicated, as outlined by Alexander and Kendall (2007), and the effects of these ligands can vary according to cell/tissue type, whether the receptor is native or overexpressed, whether allosteric modulators are present, and can display agonist bias at target sites. Some of these reasons might explain why homogenous responses are not observed to cannabinoids in the vasculature (see Table 1).

The first *in vitro* report of cannabinoid-induced vasorelaxation conducted by Ellis *et al.* (1995) showed that the first identified endocannabinoid anandamide (AEA) and the plant-derived psychotropic cannabinoid Δ^9-tetrahydrocannabinol (THC) cause vasorelaxation of rabbit cerebral arteries associated with an increase in vasoactive prostanoids. Since then, many studies have shown and characterized the vasorelaxant effects of a range of cannabinoids (see Tables 1 and 2 for a summary of the current knowledge in animal and human studies respectively). There is also evidence that cannabinoids cause vasoconstrictor responses in some vascular beds. Mechanistic studies have identified the involvement of numerous receptors, ion channels and vasoactive products of cannabinoid metabolism. The direct vascular effects of cannabinoids appear dependent on the cannabinoid, the arterial bed and the experimental preparation, that is whether the studies are using isolated arteries or a whole perfused vascular bed. There may also be potential species differences in responses.

The aim of this review is to examine the evidence in experimental and human studies, indicating the involvement of a range of receptors in cannabinoid-mediated responses within the vasculature, including CB_1, CB_2, CB_e, GPR55, TRPs, PPARs, $5\text{-}HT_{1A}$ and prostanoid receptors. A further aim of this review is to address whether other cannabinoid receptors in the vasculature have yet to be identified and what other non-cannabinoid target sites might cannabinoids act at. This review will not discuss the cardiovascular effects of cannabinoids *in vivo*, where modulation of the autonomic nervous system at a presynaptic level appears to be the dominant mechanism of action in altering haemodynamics (see Malinowska *et al.*, 2012).

CB_1

Animal studies

A potential role for CB_1 activation is one of the most commonly investigated mechanisms of action for the vascular effects of cannabinoids. Many studies have shown that the vasorelaxant response to cannabinoids including AEA, N-arachidonoyl dopamine (NADA), oleoylethanolamide (OEA), oleamide, CP55,940, WIN55212-2 and HU-210 in a range of different arterial preparations including renal, mesenteric, opthalmic and cerebral arteries is mediated at least in part by activation of the CB_1 receptor (see Table 1 for details, only studies where an effect of the antagonist was observed at concentrations ≤1 μM are included because non-CB_1 effects of CB_1 antagonists such as SR141716A can exist at higher concentrations).

Despite the wealth of evidence implicating CB_1 in the vascular responses to cannabinoids, a significant number of studies have not revealed a role for CB_1 activation, sometimes even with the same agonist, in the same arterial bed, or in the same species. For example, vasorelaxation to AEA is sensitive to CB_1 receptor antagonism in renal arterioles (Deutsch *et al.*, 1997; Koura *et al.*, 2004), rat mesenteric arteries (White and Hiley, 1998; O'Sullivan *et al.*, 2004a), the perfused mesenteric bed (Wagner *et al.*, 1999), bovine ophthalmic arteries (Romano and Lograno, 2006), cat cerebral arteries (Gebremedhin *et al.*, 1999) and rabbit aorta (Mukhopadhyay *et al.*, 2002). However, CB_1 antagonism does not affect AEA-induced vasorelaxation in rat mesenteric arteries (Plane *et al.*, 1997), the rat mesenteric bed (Peroni *et al.*, 2004), rat hepatic arteries or guinea pig basilar arteries (Zygmunt *et al.*, 1999), or the rat aorta (O'Sullivan *et al.*, 2005b). AEA is also capable of causing vasorelaxation of the same magnitude in the mesenteric bed of $CB_1^{+/+}$ as $CB_1^{-/-}$ mice (Jarai *et al.*, 1999). Furthermore, CB_1 receptor antagonism does not affect the vasorelaxant effect of THC, a CB_1 agonist, in rat hepatic arteries (Zygmunt *et al.*, 2002), rat mesenteric arteries (Zygmunt *et al.*, 2002; O'Sullivan *et al.*, 2005b) and the rat aorta (O'Sullivan *et al.*, 2005c). Interestingly, Wagner *et al.* (1999) found that although AEA relaxes the whole mesenteric bed sensitive to CB_1 antagonism, in the same study, other CB_1 agonists including WIN55212-2, HU-210, THC and 2-arachidonoylglycerol (2-AG) did not. Similarly, WIN55212-2 failed to produce vasorelaxation of the rabbit aorta although vasorelaxant to methanandamide or AEA in the same study was sensitive to CB_1 antagonism (Mukhopadhyay *et al.*, 2002). It seems unusual that if CB_1 receptors are present and can cause vasorelaxation, then this would not be observed with all CB_1 agonists. WIN55212-2 can relax other arteries such as the rat middle cerebral artery (Rademacher *et al.*, 2005) and rat aorta (Dannert *et al.*, 2007) sensitive to CB_1 antagonism, so it can at least functionally couple to CB_1 with regard to vasorelaxation in some arteries.

It is difficult to tell what the discrepancies for these results might be. Certainly, some will be due to whether or not the CB_1 receptor is expressed in a given segment of artery. For example, AEA does not relax rat distal femoral arteries, and there is no detectable CB_1 in these arteries (Domenicali *et al.*, 2005). Another possibility is that the antagonists used in these studies are acting at receptors other than CB_1. It is already known that SR141716A can antagonize the CB_e receptor at concentration greater than 1 μM (Jarai *et al.*, 1999) and that SR141716A has vascular effects unrelated to CB_1 such as inhibition of gap junctions and Ca^{2+}-induced relaxation (Chaytor *et al.*, 1999; Bukoski *et al.*, 2002). Less is known about non-CB_1 actions of AM251 (although structurally very similar to SR141716A), but off-target effects of these

Table 1

Summary of known sites of action for cannabinoids and similar chemicals in the vasculature in animal studies

	CB_1*	CB_2	Endothelium and CB_e	Sensory nerves (TRPV1)	Nuclear receptors	Other receptors
AEA	Vasorelaxation sensitive to CB_1 antagonism in renal arterioles (Deutsch *et al.*, 1997; Koura *et al.*, 2004), rat mesenteric arteries (White and Hiley, 1998; O'Sullivan *et al.*, 2004a), the perfused mesenteric bed (Wagner *et al.*, 1999), bovine ophthalmic arteries (Romano and Lograno, 2006) and rabbit aorta (Mukhopadhyay *et al.*, 2002). CB_1 antagonism partially inhibited time-dependent (1 h) vasorelaxation in the bovine ophthalmic artery (Romano and Lograno, 2012). CB_1 antagonism inhibited increased COX-2 expression in mouse cerebral endothelial cells (Chen *et al.*, 2005) and release of NO in rat mesenteric bed (Poblete *et al.*, 2005). No role for CB_1 in vasorelaxation of rat aorta (O'Sullivan *et al.*, 2005a; Herradón *et al.*, 2007), rat mesenteric bed (Harris *et al.*, 2002; Peroni *et al.*, 2004), rat small mesenteric arteries (Ho and Hiley, 2003), rabbit mesenteric arteries (Kagota *et al.*, 2001), rat coronary arteries (White *et al.*, 2001), rat hepatic (Zygmunt *et al.*, 1999), rat pulmonary artery (Baranowska-Kuczko *et al.*, 2012), bovine coronary arteries (Pratt *et al.*, 1998) or guinea pig basilar arteries (Zygmunt *et al.*, 1999).	No role for CB_2 in vasorelaxation in the rat aorta (O'Sullivan *et al.*, 2005b; Herradón *et al.*, 2007), rabbit aorta (Mukhopadhyay *et al.*, 2002), rat coronary arteries (White *et al.*, 2001), rat pulmonary artery (Baranowska-Kuczko *et al.*, 2012), rat mesenteric arteries (Ho and Hiley, 2003). No role for CB_2 in time-dependent responses in the rat aorta (O'Sullivan *et al.*, 2009a).	Vasorelaxation sensitive to removal of the endothelium in bovine coronary arteries (Pratt *et al.*, 1998), bovine ophthalmic arteries (Romano and Lograno, 2006), rat mesenteric bed (Wagner *et al.*, 1999), rat mesenteric arteries (O'Sullivan *et al.*, 2004a), rat aorta (Herradón *et al.*, 2007), rat pulmonary artery (Baranowska-Kuczko *et al.*, 2012), sheep coronary artery (Grainger and Boachie-Ansah, 2001). Vasorelaxation sensitive to O-1918 in rat small mesenteric arteries (O'Sullivan *et al.* 2004a), the rat aorta (Herradón *et al.*, 2007) and rat pulmonary artery (Baranowska-Kuczko *et al.*, 2012). No role for CB_e in vasorelaxation of the rat superior mesenteric artery (O'Sullivan *et al.* 2004a), rat mesenteric arteries (White and Hiley, 1998; Ho and Hiley, 2003), rabbit mesenteric arteries (Kagota *et al.*, 2001), rat coronary arteries (White *et al.*, 2001) or rat aorta (O'Sullivan *et al.*, 2005b).	Vasorelaxation inhibited by capsaicin or capsazepine in rat mesenteric arteries (Zygmunt *et al.*, 1999; Harris *et al.*, 2002; Ho and Hiley, 2003; O'Sullivan *et al.* 2004a; Peroni *et al.*, 2004), rat hepatic arteries (Zygmunt *et al.*, 1999) and guinea pig basilar arteries (Zygmunt *et al.*, 1999). Release of NO in rat mesenteric bed inhibited by TRPV1 antagonists (Poblete *et al.*, 2005). No role for sensory nerves in vasorelaxation of the rat aorta (O'Sullivan *et al.*, 2005a; Herradón *et al.*, 2007), rat pulmonary artery (Baranowska-Kuczko *et al.*, 2012), rat coronary arteries (White *et al.*, 2001), sheep coronary artery (Grainger and Boachie-Ansah, 2001).	Time-dependent response in the bovine ophthalmic artery inhibited by PPARα antagonism (Romano and Lograno, 2012). Time-dependent response in the rat aorta inhibited by PPARγ antagonism (O'Sullivan *et al.*, 2009a).	Vasorelaxation sensitive to CGRP receptor antagonism (Zygmunt *et al.*, 1999). Vasorelaxation of rat pulmonary artery sensitive to prostacyclin (IP) receptor antagonism (Baranowska-Kuczko *et al.*, 2012). Vasoconstrictor effects in the rabbit lung inhibited by EP_1 receptor antagonism (Wahn *et al.*, 2005).

Table 1

Continued

	CB_1*	CB_2	Endothelium and CB_e	Sensory nerves (TRPV1)	Nuclear receptors	Other receptors
2-AG	No role for CB_1 in vasorelaxation of bovine coronary arteries (Gauthier *et al.*, 2005), rat aorta (Stanke-Labesque *et al.*, 2004) or rabbit mesenteric arteries (Kagota *et al.*, 2001).	No role for CB_2 in rat aorta (Stanke-Labesque *et al.*, 2004).	Vasorelaxation of bovine coronary arteries inhibited by endothelium denudation (Gauthier *et al.*, 2005). No role for endothelium in rabbit mesenteric arteries (Kagota *et al.*, 2001).	N/A	N/A	Vasoconstrictor effect in rat aorta inhibited by thromboxane receptor antagonist (Stanke-Labesque *et al.*, 2004).
NADA	Vasorelaxation in rat small and large mesenteric arteries inhibited by SR141716A or AM251 (O'Sullivan *et al.*, 2004b). Time-dependent, but not acute, response in the rat aorta inhibited by AM251 (O'Sullivan *et al.*, 2005b; O'Sullivan *et al.*, 2009a).	No role for CB_2 in acute (O'Sullivan *et al.*, 2005b) or time-dependent response in the rat aorta (O'Sullivan *et al.*, 2009a).	Vasorelaxation sensitive to O-1918 in rat small mesenteric arteries (O'Sullivan *et al.*, 2004b).	Vasorelaxation inhibited by capsaicin/ capsazepine in rat small and large mesenteric arteries (O'Sullivan *et al.*, 2004b), but not in the aorta (O'Sullivan *et al.*, 2005b).	Time-dependent response in the rat aorta inhibited by GW9662 (O'Sullivan *et al.*, 2009a).	N/A
OEA	Vasorelaxation inhibited by AM251 in rat small mesenteric arteries (Suleimani and Hiley, 2013) and the aorta, but not whole mesenteric bed (Wheal *et al.*, 2010).	Vasorelaxation inhibited by AM630 in rat small mesenteric arteries (Suleimani and Hiley, 2013).	Vasorelaxation sensitive to removal of the endothelium and O-1918 in rat small mesenteric arteries (Suleimani and Hiley, 2013), whole mesenteric bed and aorta (Wheal *et al.*, 2010).	Inhibited by capsaicin in rat small mesenteric arteries (Ho *et al.*, 2008; Suleimani and Hiley, 2013), the whole mesenteric bed and aorta (Wheal *et al.*, 2010).	N/A	Vasoconstrictor metabolites of OEA acting through thromboxane A2 receptor in rat mesenteric arteries (Wheal *et al.*, 2010)
PEA	No role for CB_1 in time-dependent vasorelaxation of the bovine ophthalmic artery (Romano and Lograno, 2012).	N/A	No role for CB_e in vasorelaxation of rat small mesenteric arteries (White and Hiley, 1998).	Inhibited by capsaicin (but not capsazepine) in rat small mesenteric arteries (Ho *et al.*, 2008).	Time-dependent response in the bovine ophthalmic artery inhibited by GW6471 (Romano and Lograno, 2012).	N/A

Oleamide	Evidence of both a role for CB_1 (Sudhahar *et al.*, 2009) and no role (Hoi and Hiley, 2006) in vasorelaxation of rat small mesenteric arteries.	No role for CB_2 in relaxation of rat small mesenteric arteries (Hoi and Hiley, 2006; Sudhahar *et al.*, 2009)	Vasorelaxation sensitive to removal of the endothelium and O-1918 in rat small mesenteric arteries (Hoi and Hiley, 2006; Sudhahar *et al.*, 2009) but not in the rat aorta (Hopps *et al.*, 2012).	Vasorelaxation inhibited by capsaicin or capsazepine in rat small mesenteric arteries (Hoi and Hiley, 2006; Sudhahar *et al.*, 2009) and by capsaicin in the rat aorta (Hopps *et al.*, 2012)	N/A	N/A
ARA-S	No role for CB_1 in vasorelaxation of rat aorta (Milman *et al.*, 2006).	No role for CB_2 in vasorelaxation of rat aorta (Milman *et al.*, 2006).	Vasorelaxation in rat aorta sensitive to removal of the endothelium but not O-1918 (Milman *et al.*, 2006). Vasorelaxation in rat mesenteric sensitive to removal of the endothelium and O-1918 (Milman *et al.*, 2006; Godlewski *et al.*, 2009).	N/A	N/A	Vasorelaxation of rat aorta inhibited by PTX but not O-1918 (Milman *et al.*, 2006).
NAGly	No role for CB_1 in vasorelaxation of rat mesenteric arteries (Parmar and Ho, 2010).	No role for CB_2 in vasorelaxation of rat mesenteric arteries (Parmar and Ho, 2010).	Vasorelaxation in rat mesenteric arteries sensitive to removal of the endothelium and O-1918 (Parmar and Ho, 2010).	No role in vasorelaxation of rat mesenteric arteries (Parmar and Ho, 2010).	N/A	N/A
THC	No role for CB_1 in relaxation of small mesenteric arteries (Zygmunt *et al.*, 2002; O'Sullivan *et al.*, 2005b) and rat hepatic arteries (Zygmunt *et al.*, 2002). Vasoconstriction, but not vasorelaxation, sensitive to SR141716A in the rat aorta (O'Sullivan *et al.*, 2005a) and the superior mesenteric artery (O'Sullivan *et al.*, 2005b).	Vasorelaxation of rat aorta inhibited by SR144528 (O'Sullivan *et al.*, 2005a).	No role for the endothelium in vasorelaxation of small mesenteric arteries (Zygmunt *et al.*, 2002; O'Sullivan *et al.*, 2005b) and rat hepatic arteries (Zygmunt *et al.*, 2002). Vasorelaxation of rat aorta sensitive to removal of the endothelium (O'Sullivan *et al.*, 2005a).	Vasorelaxation inhibited by capsaicin in rat hepatic and mesenteric arteries (Zygmunt *et al.*, 2002), rat aorta (O'Sullivan *et al.*, 2005a). No role for sensory nerves in relaxation of small mesenteric arteries (O'Sullivan *et al.*, 2005b).	N/A	Vasorelaxation sensitive to CGRP receptor antagonism (Zygmunt *et al.*, 2002). Vasorelaxation of small mesenteric arteries inhibited by PTX but not AM251 or endothelium removal (O'Sullivan *et al.*, 2005b).

Table 1

Continued

	CB_1*	CB_2	Endothelium and CB_e	Sensory nerves (TRPV1)	Nuclear receptors	Other receptors
CBD	N/A	N/A	CBD antagonizes the vasorelaxant effects of Abn-CBD in rat mesenteric arteries, suggested to be antagonism of CBe (Jarai *et al.*, 1999). Antagonizes vasorelaxant effects of AEA in rat pulmonary artery (Baranowska-Kuczko *et al.*, 2012).	N/A	Time-dependent vasorelaxation in the rat aorta inhibited by GW9662 (O'Sullivan *et al.*, 2009b).	N/A
Abn-CBD	Vasorelaxation of perfused rat mesenteric bed inhibited by SR141716A (Jarai *et al.*, 1999). No role for CB_1 in the rat pulmonary artery (Baranowska-Kuczko *et al.*, 2012).	No role for CB_2 in the rat pulmonary artery (Baranowska-Kuczko *et al.*, 2012).	Vasorelaxation of perfused rat mesenteric vascular bed (Jarai *et al.*, 1999), rat mesenteric arteries (Begg *et al.*, 2003; Offertaler *et al.*, 2003) and rat pulmonary artery (Baranowska-Kuczko *et al.*, 2012) inhibited by removal of the endothelium. Vasorelaxation sensitive to 0-1918 in rat mesenteric arteries (Offertaler *et al.*, 2003) and rat pulmonary artery (Baranowska-Kuczko *et al.*, 2012).	No role for sensory nerves in the rat pulmonary artery (Baranowska-Kuczko *et al.*, 2012).	N/A	N/A
CP55940	Vasorelaxation inhibited by SR141716A in rat small mesenteric arteries (White and Hiley, 1998; O'Sullivan *et al.* 2004a).	Vasorelaxation of rat aorta inhibited by SR144528 (O'Sullivan *et al.*, 2005b).	No role for CB_e in rat small mesenteric arteries (White and Hiley, 1998).	No role for sensory nerve activation in rat hepatic and guinea pig basilar arteries (Zygmunt *et al.*, 1999).	N/A	N/A

WIN55212-2	Vasorelaxation sensitive to CB_1 antagonism in bovine ophthalmic arteries (Romano and Lograno, 2006) and cat cerebral arteries (Gebremedhin *et al.*, 1999). Vasodilatation absent in CB1 knockout mice (Szekeres *et al.*, 2012). No role in vasorelaxation of rat small mesenteric arteries (White and Hiley, 1998; Ho and Hiley, 2003) or rat aorta (Dannert *et al.*, 2007).	Vasorelaxation of rat aorta inhibited by SR144528 (Dannert *et al.*, 2007). No role in vasorelaxation of rat small mesenteric arteries (Ho and Hiley, 2003)	No role for CB_e in rat small mesenteric arteries (White and Hiley, 1998; Ho and Hiley, 2003). Vasorelaxation of rat aorta (Dannert *et al.*, 2007) and bovine ophthalmic arteries (Romano and Lograno, 2006) inhibited by removal of the endothelium.	Vasorelaxation inhibited by capsaicin in rat aorta (Dannert *et al.*, 2007) and rat mesenteric arteries (Ho and Hiley, 2003).	N/A	Vasorelaxation of rat aorta inhibited by CGRP antagonist (Dannert *et al.*, 2007).
HU210	Vasorelaxation inhibited by SR141716A in rat small mesenteric arteries (White and Hiley, 1998)	N/A	No role for CB_e in rat small mesenteric arteries (White and Hiley, 1998).	N/A	N/A	N/A
JWH015	No role for CB_1 in rat small mesenteric arteries (Ho and Hiley, 2003)	No role for CB_2 in rat small mesenteric arteries (Ho and Hiley, 2003).	No role for CB_e in rat small mesenteric arteries (White and Hiley, 1998).	Vasorelaxation inhibited by capsaicin in rat mesenteric arteries (Ho and Hiley, 2003).	N/A	N/A

In most studies, concentration–response curves to cannabinoids (up to high μM concentrations) were used in the vascular preparation. *Only studies using receptor antagonists ≤1 μM were included. N/A, no available information.

Table 2

Summary of known sites of action for cannabinoids and similar chemicals in the human vasculature

	CB_1	CB_2	CB_e	TRP channels	PPARs	Other
Anandamide	Vasorelaxation in mesenteric arteries inhibited by AM251 (Stanley and O'Sullivan, 2012). Increase in Ca^{2+} flux in cerebromicrovascular endothelial cells is sensitive to SR141716A (Golech *et al.*, 2004). No role for CB_1 in vasorelaxation in pulmonary arteries (Kozlowska *et al.*, 2007).	No role for CB_2 in vasorelaxation in mesenteric arteries (Stanley and O'Sullivan, 2012) or pulmonary arteries (Baranowska-Kuczko *et al.*, 2013). Increase in Ca^{2+} flux in cerebromicrovascular endothelial cells is sensitive to SR144528 (Golech *et al.*, 2004).	Vasorelaxation in mesenteric arteries (Stanley and O'Sullivan, 2012) and pulmonary arteries (Baranowska-Kuczko *et al.*, 2013) is inhibited by O-1918.	Increase in Ca^{2+} flux in cerebromicrovascular endothelial cells inhibited by capsazepine (Golech *et al.*, 2004). No role for TRP in vasorelaxation of mesenteric arteries (Stanley and O'Sullivan, 2012) or pulmonary arteries (Baranowska-Kuczko *et al.*, 2013).	No role for PPARγ in vasorelaxation of small mesenteric arteries (Stanley and O'Sullivan, 2014)	Vasorelaxation in pulmonary arteries inhibited IP receptor antagonists (Baranowska-Kuczko *et al.*, 2013).
2-AG	Increase in Ca^{2+} flux in cerebromicrovascular endothelial cells (Golech *et al.*, 2004) and NO production in saphenous vein (Stefano *et al.*, 2000) is sensitive to SR141716A. No role for CB_1 in vasorelaxation in mesenteric arteries (Stanley and O'Sullivan, 2014).	Increase in Ca^{2+} flux in cerebromicrovascular endothelial cells is sensitive to SR144528 (Golech *et al.*, 2004). No role for CB_2 in vasorelaxation of mesenteric arteries (Stanley and O'Sullivan, 2014) or in NO production in saphenous vein (Stefano *et al.*, 2000).	No role for CB_e in vasorelaxation of mesenteric arteries (Stanley and O'Sullivan, 2014).	Increase in Ca^{2+} flux in cerebromicrovascular endothelial cells is sensitive to capsazepine (Golech *et al.*, 2004). No role for TRP in vasorelaxation of mesenteric arteries (Stanley and O'Sullivan, 2014).	N/A	Vasorelaxant metabolites acting through EP_4 and IP receptors in mesenteric artery (Stanley and O'Sullivan, 2014).
Virodhamine	No role for CB_1 in vasorelaxation in pulmonary arteries (Kozlowska *et al.*, 2008).	No role for CB_2 in vasorelaxation of pulmonary arteries (Kozlowska *et al.*, 2008).	Vasorelaxation in pulmonary arteries inhibited by O-1918 (Kozlowska *et al.*, 2008).	No role for TRP in vasorelaxation of pulmonary arteries (Kozlowska *et al.*, 2008).	N/A	N/A
CBD	Vasorelaxation in mesenteric arteries inhibited by AM251 (100 nM) (Stanley and O'Sullivan, 2011).	No role for CB_2 in vasorelaxation of mesenteric arteries (Stanley and O'Sullivan, 2011).	No role for CB_e in vasorelaxation of mesenteric arteries (Stanley and O'Sullivan, 2011).	Vasorelaxation in mesenteric arteries inhibited by capsaicin (10 μM) (Stanley and O'Sullivan, 2011).	No role for PPARγ in vasorelaxation of small mesenteric arteries (Stanley and O'Sullivan, 2014).	N/A
Abn-CBD	N/A	N/A	Vasorelaxation in pulmonary arteries (Kozlowska *et al.*, 2007) and cell signalling pathways in HUVECs (Offertaler *et al.*, 2003) inhibited by O-1918.	N/A	N/A	N/A

antagonists might cloud their interpretation. It should also be considered that because cannabinoids act via multiple pathways, blocking one site of action may be compensated for by other pathways. Another explanation is that cannabinoid agonists may express functional selectivity at CB_1 in the vasculature, such that some couple more effectively than others to the mechanisms that bring about relaxation such as Ca^{2+} channel inhibition and potassium channel activation. A similar phenomenon has been reported with CB_2, where some CB_2 ligands (CP55,940, JWH-015, JWH133 and 2-AG), but not others (WIN55212-2 or THC) inhibit Ca^{2+} channels through CB_2 activation (Atwood *et al.*, 2012).

When involved, the mechanism of how CB_1 activation brings about relaxation is likely to involve numerous pathways. Gebremedhin *et al.* (1999) showed that AEA and WIN55212-2 decrease Ca^{2+} currents via CB_1 in smooth muscles cells from cat cerebral microvasculature, suggesting a role for Ca^{2+} channel inhibition. However, it has been suggested that the CB_1 receptor is not linked to potassium channel activation as Romano and Lograno (2006) found that co-incubation with a potassium channel blocker and a CB_1 antagonist produced further inhibition than either inhibitor alone in the bovine ophthalmic artery. Su and Vo (2007) showed that noladin ether increases ERK 1/2 activation via CB_1 and brings about vasorelaxation. Other studies have shown that CB_1 activation in the vasculature is coupled to NO release (Deutsch *et al.*, 1997; Poblete *et al.*, 2005). In endothelial cells, stimulation of the CB_1 receptor has been reported to increase COX-2 expression (Chen *et al.*, 2005), an enzyme capable of producing both vasorelaxant and vasoconstrictor mediators. A recent study found that the vasoconstrictor effects of angiotensin II can be enhanced by antagonism of the CB_1 receptor or inhibition of 2-AG synthesis (Szekeres *et al.*, 2012). Similarly, contractions in the rat middle cerebral artery to U46619 could be enhanced by antagonism of the CB_1 receptor (Rademacher *et al.*, 2005). Therefore, the vascular effects of cannabinoids via CB_1 may not be restricted to direct vasorelaxant effects, but may also depend on their ability to decrease the effects of vasoconstrictors.

Human studies

The expression of the CB_1 receptor has been confirmed in human endothelial cell lines (Liu *et al.*, 2000) and vascular smooth muscle cell lines (Sugiura *et al.*, 1995). In human brain endothelial cells (HBECs), the CB_1 receptor is expressed and contributes to AEA and 2-AG-induced increases in intracellular Ca^{2+} (Golech *et al.*, 2004). In human isolated mesenteric arteries, AEA and cannabidiol (CBD)-induced vasorelaxation are both inhibited by CB_1 antagonism (Stanley and O'Sullivan, 2011; 2012). However, in the same arteries, the vasorelaxant effect of 2-AG was not CB_1 mediated (Stanley *et al.*, 2011), even though 2-AG is a potent agonist at the CB_1 receptor (Mechoulam *et al.*, 1995), causes CB_1-mediated vasorelaxation in the same arterial bed in other species (Kagota *et al.*, 2001) and increases NO release from human saphenous vein in a CB_1-dependent manner (Stefano *et al.*, 2000). AEA or virodhamine-induced vasorelaxation of the human pulmonary artery is also not dependent on activation of the CB_1 receptor (Kozlowska *et al.*, 2007; 2008). The role for CB_1 in human vasculature thus far seems similar to that observed in animal studies, with evidence both for and against a role for CB_1 depending on the agonist and artery studied.

CB_2

Animal studies

When investigated, most studies have found that there is no involvement of the CB_2 receptor in mediating the vascular responses to cannabinoids in animal studies (see Table 1). In rat mesenteric resistance arteries, the CB_2 receptor agonist JWH-015 causes vasorelaxation; however, this was not inhibited by CB_2 antagonism (Ho and Hiley, 2003). AlSuleimani and Hiley (2013) did show a role for CB_2 in the OEA-induced vasorelaxation of small resistance arteries of the mesenteric bed, but this is the only study to suggest a role for CB_2 in mesenteric arteries. When a role for CB_2 has been observed, it has mainly been in the rat aorta, where the vasorelaxant effects of THC, CP55,940 and WIN55,212-2 were partially inhibited by CB_2 antagonism (O'Sullivan *et al.*, 2005b; Dannert *et al.*, 2007). AEA and HU-210 also induce vasorelaxation that is inhibited by CB_2 antagonism in rat coronary arteries (Mair *et al.*, 2010). This might reflect regional variations in the role of the CB_2 receptor in the vasculature, but in general, it can be concluded that CB_2 activation is not the main mechanism that brings about the vasorelaxant effects of cannabinoids in animals.

Human studies

CB_2 receptor expression has been confirmed in HBECs (Golech *et al.*, 2004; Schley *et al.*, 2009; Ramirez *et al.*, 2012), human coronary artery endothelial and smooth muscle cell lines (Rajesh *et al.*, 2007; 2008b). In HBECs, CB_2 receptor activation via AEA and 2-AG increases Ca^{2+} influx (Golech *et al.*, 2004), and in human coronary endothelial cells, CB_2 activation decreases TNF-α-induced endothelial activation, transendothelial migration of monocytes and vascular adhesion molecules (Rajesh *et al.*, 2007). In human mesenteric arteries, CB_2 antagonism does not affect the vasorelaxant responses to CBD, AEA or 2-AG (Stanley *et al.*, 2011; Stanley and O'Sullivan, 2011; 2012). Furthermore, the CB_2 agonist HU308 does not cause vasorelaxation of these arteries (Stanley and O'Sullivan, 2014). Vasorelaxation to virodhamine and AEA in human pulmonary arteries is also not inhibited by CB_2 antagonism (Kozlowska *et al.*, 2008; Baranowska-Kuczko *et al.*, 2013). Taken together, this suggests that the CB_2 receptor is expressed in human vascular tissue, but does not play a role in mediating the vasorelaxant response to cannabinoids, as also suggested through numerous animal studies (see Table 1). It is more likely that CB_2 plays a role in other functions of the human endothelium such as the regulation of adhesion molecules, monocyte adhesion and endothelial permeability (Rajesh *et al.*, 2007; Ramirez *et al.*, 2012).

CB_e

Animal studies

Early indications of an endothelial cannabinoid receptor that is distinct from CB_1 and CB_2 came from the works of Jarai

et al. (1999) who showed that AEA, methanandamide and abnormal cannabidiol (Abn-CBD) were able to cause vasodilatation of the mesenteric vasculature equally in $CB_1^{-/-}$, $CB_2^{-/-}$ as in wild-type mice. This vasodilatation was inhibited by removal of the endothelium and in the presence of higher (> μM) concentrations of SR141716A (in $CB_1^{-/-}$ animals). This work suggested the involvement of receptors other than CB_1 or CB_2 located on the endothelium causing vasorelaxation. This has become known as the endothelial cannabinoid receptor or CB_e. The activation of this receptor by Abd-CBD and AEA has been confirmed in numerous studies (see Table 1). In rabbit aortic rings, AEA causes vasorelaxation through a pertussis toxin (PTX)-sensitive endothelial receptor (Mukhopadhyay *et al.*, 2002), and in the rat aorta, AEA-induced relaxation is sensitive to endothelium denudation, PTX and O-1918 (a proposed antagonist of CB_e that has no affinity at CB_1 or CB_2 receptors), but not CB_1 or CB_2 antagonism (Herradón *et al.*, 2007). Similar results have been obtained in rat resistance mesenteric arteries (O'Sullivan *et al.*, 2004a). In the rat mesenteric bed, Abn-CBD causes vasorelaxation that is sensitive to endothelium denudation, O-1918 and PTX (Offertaler *et al.*, 2003). Abn-CBD also stimulates NO production in rabbit aortic endothelial cells which was antagonized by O-1918 and PTX but not CB_1 or CB_2 (McCollum *et al.*, 2007). In the rabbit pulmonary artery, Abn-CBD causes vasorelaxation sensitive to O-1918 (Su and Vo, 2007).

Other cannabinoids that have been suggested to act through CB_e include NADA in rat mesenteric arteries (O'Sullivan *et al.*, 2004b), OEA in rat mesenteric arteries and aorta (Wheal *et al.*, 2010; AlSuleimani and Hiley, 2013), oleamide in rat mesenteric resistance arteries (Hoi and Hiley, 2006), and N-arachidonoyl-L-serine (ARA-S, Milman *et al.*, 2006) and N-arachidonoyl glycine (Parmar and Ho, 2010) in rat mesenteric arteries. However, not all cannabinoids activate the proposed CB_e receptor. The vasorelaxant effects of THC are not inhibited by removal of the endothelium in rabbit mesenteric arteries (Fleming *et al.*, 1999), rat hepatic or mesenteric arteries (Zygmunt *et al.*, 2002; O'Sullivan *et al.*, 2005a). Similarly, there is no role for CB_e in the vasorelaxation effects of 2-AG (Kagota *et al.*, 2001), palmitoylethanolamine (PEA) (White and Hiley, 1998), CP55,940 or WIN55212-2 (White and Hiley, 1998), HU-210 or JWH-015 (White and Hiley, 1998). CP55,940 and WIN55212-2 do not increase NO production in rabbit aortic endothelial cells via CB_e (McCollum *et al.*, 2007). Some of this might be explained by differences in the expression/function of the receptor, as it has been shown that while the vasorelaxant response to AEA in small mesenteric arteries involves CB_e, the same study showed there is no role for CB_e in AEA responses in the superior mesenteric artery (O'Sullivan *et al.*, 2004a).

The mechanism of how activation of CB_e brings about vasorelaxation is suggested to involve the release of an Endothelium-derived hyperpolarising factor (Jarai *et al.*, 1999; O'Sullivan *et al.*, 2004b) and involve K_{Ca} channel activity, specifically BK (Hoi and Hiley, 2006). Other studies suggest the involvement of NO production (Mukhopadhyay *et al.*, 2002; Herradón *et al.*, 2007; McCollum *et al.*, 2007), while some find no role for NO (Jarai *et al.*, 1999; Offertaler *et al.*, 2003). In rabbit aortic endothelial cells, the increase in NO production via CB_e activation has been shown to involve the activation of PI3K, Akt and phosphorylation of eNOS (McCollum *et al.*, 2007). MAPK inhibition also completely abolished the O1918-sensitive vasorelaxation to Abn-CBD (Su and Vo, 2007).

Human studies

In the human pulmonary artery and mesenteric arteries, AEA causes endothelium-dependent vasorelaxation that can be inhibited using the proposed CB_e antagonist O-1918 (Stanley and O'Sullivan, 2012; Baranowska-Kuczko *et al.*, 2013). Furthermore, in the human pulmonary artery the vasorelaxant effects of virodhamine and Abn-CBD are inhibited using O-1918 or CBD (CBD is suggested to antagonize CB_e) (Kozlowska *et al.*, 2007; 2008). These findings are similar to those reported in the same arteries in animal studies (see Table 1). In HUVECs, activation of CB_e by Abn-CBD causes activation of 42/44 MAPK, Akt and PI3K (Offertaler *et al.*, 2003; Mo *et al.*, 2004; Milman *et al.*, 2006), and modulates BK_{ca} channel activity (Begg *et al.*, 2003).

Other uncloned vascular CBs

Animal studies

Some pharmacological evidence suggests that there may be other cannabinoid receptors in the vasculature that remain to be identified. In resistance arteries of the mesenteric bed, THC-induced vasorelaxation is inhibited by PTX, but not CB_1, suggesting THC might act through an unidentified GPCR (O'Sullivan *et al.*, 2005a). THC is not sensitive to removal of the endothelium in these arteries, therefore it is unlikely to be the proposed endothelial cannabinoid receptor, and is possibly expressed on the smooth muscle. 2-AG-induced vasorelaxation of the rabbit mesenteric arteries is inhibited by 3 μM but not 1 μM SR141716A, and is not affected by removal of the endothelium, which is not consistent with a role for either CB_1 or CB_e, but a vascular smooth muscle site (Kagota *et al.*, 2001). ARA-S-induced vasorelaxation of rat mesenteric arteries is inhibited by O-1918 (even in denuded arteries) but not PTX (Milman *et al.*, 2006), which casts doubt on the specificity of actions of O-1918 at CB_e if it inhibits responses in endothelial-denuded arteries. In the rat aorta, vasorelaxation to AEA or NADA is inhibited by PTX, but not by antagonism of either CB_1 or CB_2 or removal of the endothelium (O'Sullivan *et al.*, 2005b), again suggesting a GPCR located on the smooth muscle. Similarly, vasorelaxation of rat aorta to ARA-S is inhibited by PTX but not O-1918, SR141716 or SR144528 (Milman *et al.*, 2006). Together, this suggests that further cannabinoid target sites of action on vascular smooth muscle may exist.

TRP channel activation

Animal studies

Zygmunt *et al.* (1999) first showed that the vasorelaxant effects of AEA, but not 2-AG, PEA, HU-210, WIN55,212-2 or CP55,940, could be blocked by capsaicin pretreatment (to deplete sensory neurotransmitters) or antagonized by the

transient receptor potential vanilloid 1 (TRPV1) antagonist capsazepine in rat mesenteric arteries. This then involves the release of calcitonin gene-related peptide (CGRP) causing vasorelaxation through activation of CGRP receptors (Zygmunt *et al.*, 1999). AEA-induced vasorelaxation though TRPV1 is also reported to be linked to NO production in the rat mesenteric vascular bed (Poblete *et al.*, 2005). Many studies have since confirmed the role of TRPV1 in AEA-induced vasorelaxation (Harris *et al.*, 2002; Ho and Hiley, 2003; Peroni *et al.*, 2004; O'Sullivan *et al.*, 2004a). However, in rat coronary arteries and rat pulmonary arteries, AEA-induced vasorelaxation is not affected by incubation with capsaicin and capsazepine (White *et al.*, 2001; Baranowska-Kuczko *et al.*, 2012), which may reflect differences in the sensory innervations or TRP expression between artery types.

Other cannabinoids that have been shown to cause vasorelaxation through TRPV1 activation include methanandamide (Mukhopadhyay *et al.*, 2002), NADA (O'Sullivan *et al.*, 2004b), OEA (Ho *et al.*, 2008; Wheal *et al.*, 2010; Suleimani and Hiley, 2013) and WIN55212-2 (Ho and Hiley, 2003; Dannert *et al.*, 2007). Phytocannabinoids also cause sensory-nerve-mediated vasorelaxation. In rat hepatic arteries, THC- and cannabinol-induced vasorelaxation is inhibited by capsaicin treatment and antagonism of CGRP receptors (Mukhopadhyay *et al.*, 2002). Interestingly, in mesenteric arteries from TRPV1 knockout mice, the vasorelaxation to AEA is almost completely abolished; however, the vasorelaxation to THC is only slightly reduced (Zygmunt *et al.*, 2002). This suggests that the TRPV1 receptor is the main mechanism involved in AEA-induced vasorelaxation, whereas TRPV1 only partially mediated the effects of THC. Vasorelaxation to THC was sensitive to ruthenium red, which inhibits several other members of the TRPV family. Similarly, the vasorelaxant effects of PEA are inhibited by capsaicin but not capsazepine in rat small mesenteric arteries (Ho *et al.*, 2008), and the vasorelaxant response of oleamide is inhibited by capsaicin pretreatment but not capsazepine or ruthenium red (Hopps *et al.*, 2012). This suggests that other members of the TRPV family might be responsible for the vascular effects of some cannabinoids, although this remains to be established.

Interestingly, the vasorelaxant response to AEA in mesenteric beds is greater in female than male rats, which was found to be due to an increased role for TRPV1 mediated by oestrogen (Peroni *et al.*, 2004). However, a previous study did not observe any sex differences in the vasorelaxation induced by AEA in perfused mesenteric beds (McCulloch and Randall, 1998).

Human studies

The expression of TRPV1 has been demonstrated in HBECs (Golech *et al.*, 2004) and human pulmonary artery smooth muscle cell lines (Wang *et al.*, 2008). Golech *et al.* (2004) showed that AEA, 2-AG and methanandamide cause Ca^{2+} influx through capsazepine-sensitive pathways in HBECs. Movahed *et al.* (2005) showed that intra-arterial application of AEA into human forearm microcirculation has no effect on blood flow, but dermal application of AEA increases forearm blood flow in a capsazepine-sensitive manner (suggesting TRPV1 activation). However, in isolated human mesenteric arteries and pulmonary arteries, capsaicin pretreatment or capsazepine incubation does not inhibit AEA-induced vasorelaxation (Stanley and O'Sullivan, 2012; Baranowska-Kuczko *et al.*, 2013). Furthermore, 2-AG- and virodhamine-induced vasorelaxation in human mesenteric and pulmonary arteries was not inhibited by either capsaicin pretreatment or capsazepine incubation (Kozlowska *et al.*, 2008; Stanley *et al.*, 2011). However, CBD-induced vasorelaxation was inhibited by capsaicin pretreatment in human mesenteric arteries (Stanley and O'Sullivan, 2011) and a vasorelaxant response to capsaicin has also been observed in these arteries (Stanley and O'Sullivan, 2014). Therefore, in human mesenteric arteries, this would suggest the presence of functional TRPV receptors, which are not activated by AEA and 2-AG, but are activated by CBD. This is different to what has been shown in the same arteries in animal studies where TRPV1 is involved in AEA-mediated vascular responses.

GPR55

Animal studies

GPR55 is an orphan GPCR widely expressed in animal tissues (Godlewski *et al.*, 2009). GPR55 is expressed in whole arteries (Daly *et al.*, 2010), and the proposed endogenous ligand at this receptor is lysophosphatidylinositol (LPI, Liu *et al.*, 2009). Cannabinoids are also suggested to be ligands at this receptor (see Gasperi *et al.*, 2013, for a recent review). Ryberg *et al.* (2007) showed that 2-AG, PEA, CP55,940, THC and AM251 stimulate GTPγS binding in GPR55-transfected HEK 293 cells. Lauckner *et al.* (2008) also showed the binding of THC, JWH-015, AEA and methanandamide in GPR55-transfected HEK 293 cells. However, another study in GPR55-transfected HEK 293 cells suggested AEA and 2-AG were not able to stimulate calcium signalling (Henstridge *et al.*, 2009).

It was initially suggested that GPR55 might be the proposed endothelial cannabinoid receptor CB_e. However, there is now evidence against this suggestion, as mesenteric arteries of both wild-type and GPR55 knockout mice show vasorelaxation in the presence of Abn-CBD and O-1602 (an analogue of Abn-CBD), which is sensitive to O-1918 (Johns *et al.*, 2007). Despite this, activation of GPR55 by cannabinoids may still play a role in the vasculature since a recent oral communication suggested GPR55 knockout mice showed reduced vasorelaxation to AEA in GPR55 knockout mice (McNaughton and Ho, 2013).

Human studies

Primary human dermal microvascular endothelial cells and HUVECs express GPR55 (Corriu *et al.*, 1996). In human endothelial cells, AEA stimulates both CB_1 and GPR55, producing different intracellular signalling pathways; CB_1 is $G_{i/o}$ coupled while GPR55 is coupled to G_q (Waldeck-Weiermair *et al.*, 2008). Knockdown of GPR55 in human endothelial cells partly inhibited the ARA-S-induced angiogenesis and endothelial wound healing, suggesting a role for GPR55 in the vasculature (Zhang *et al.*, 2010). LPI stimulation of GPR55 also results in BK_{Ca} activation and subsequent membrane hyperpolarization in endothelial cells (Bondarenko *et al.*, 2010), a key pathway involved in vasorelaxation.

PPARs

Animal studies

THC causes time-dependent and PPARγ-dependent vasorelaxation in rat-isolated arteries (the aorta and superior mesenteric artery) that is dependent on NO and hydrogen peroxide (H_2O_2) production and superoxide dismutase activity (O'Sullivan *et al.*, 2005c). Furthermore, 2 h incubation with THC *in vitro* enhances subsequent vasodilator responses to acetylcholine in isolated arteries, which was also inhibited by a PPARγ antagonist (O'Sullivan *et al.*, 2006). A similar time-dependent and PPARγ-sensitive vasorelaxant response was seen to CBD (O'Sullivan *et al.*, 2009b) and the endocannabinoids AEA and NADA, but not PEA (O'Sullivan *et al.*, 2009a). Romano and Lograno (2012) have also recently shown a similar time-dependent vasorelaxant response to AEA and PEA in the bovine ophthalmic artery that could be inhibited by a PPARα, but not PPARγ, antagonist. Together, these data suggest that time-dependent PPAR-mediated responses to cannabinoids are also observed in the vasculature.

Human studies

PPARα, PPARγ and PPARδ are expressed in a range of human arterial smooth muscle and endothelial cells (Inoue *et al.*, 1998; Staels *et al.*, 1998; Marx *et al.*, 1999; Law *et al.*, 2000; Yang *et al.*, 2002; Kim *et al.*, 2011). In HUVECs, 2-AG and AEA increase luciferase transcriptional activity (indirectly through COX-2) at PPARδ but not PPARα (Ghosh *et al.*, 2007). Through this pathway, 2-AG decreases prothrombotic mediators (Ghosh *et al.*, 2007). In a viral model of multiple sclerosis, WIN55212-2 suppresses the increase in intercellular cell adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) in brain endothelium, sensitive to PPARγ antagonism, but not CB_1 or CB_2 antagonism (Mestre *et al.*, 2009). An analogue of OEA has also been shown to decrease the expression of VCAM and ICAM and monocyte adhesion in response to inflammation in HUVECs mediated by PPARα (Chen *et al.*, 2011).

In isolated mesenteric resistance arteries, we recently showed that AEA and CBD cause time-dependent vasorelaxation (Stanley and O'Sullivan, 2014), similar to PPARγ-mediated vasorelaxation seen in animal arteries (O'Sullivan *et al.*, 2005c). However, in human mesenteric arteries, the time-dependent effects of AEA and CBD were not inhibited by PPARγ antagonism. However, in rat arteries, cannabinoid activation of PPARγ receptors was exclusive to conduit arteries and absent in small resistance arteries (O'Sullivan *et al.*, 2006). Therefore, the resistance arteries used in the human arteries may be too small to elicit a PPARγ-mediated response.

5-HT_{1A}

A range of 5-HT receptor subtypes are expressed in the cardiovascular system (Villalon and Centurion, 2007); however, the role (vasorelaxant/vasoconstrictor) that these receptors play in the regulation of vascular tone varies (Watts and Davis, 2011). In non-vascular tissues, phytocannabinoids and synthetic cannabinoids have been shown to activate (Russo *et al.*, 2005), antagonize (Cascio *et al.*, 2010) and increase the expression (Zavitsanou *et al.*, 2010) of the 5-HT_{1A} receptor. In the vasculature, CBD increases cerebral artery blood flow *in vivo* and causes reductions in infarct damage caused by cerebral artery occlusion, which was inhibited by a 5-HT_{1A} antagonist (Mishima *et al.*, 2005). In mice, *in vivo* CBD also reduces stress-induced hypertension which can be inhibited by a 5-HT_{1A} antagonist (Resstel *et al.*, 2009).

Receptor targets for metabolic products of cannabinoids

Many studies have shown that some of the vascular effects of some cannabinoids are mediated by their metabolic products. The vasorelaxant effects of THC, AEA and 2-AG can be inhibited by fatty acid amide hydrolase, MAGL, COX and cytochrome p450 inhibition (Kaymakcalan and Turker, 1975; Ellis *et al.*, 1995; Fleming *et al.*, 1999; Gauthier *et al.*, 2005; Herradón *et al.*, 2007; Awumey *et al.*, 2008; Czikora *et al.*, 2012). The metabolites shown to be produced within the vasculature include arachidonic acid, prostaglandins and epoxyeicosatrienoic acids (Pratt *et al.*, 1998; Stanke-Labesque *et al.*, 2004; Chen *et al.*, 2005), which can themselves have direct vascular effects or further be metabolized into vasoactive substances. Some studies have investigated the receptors that those metabolic products might be acting at. Kaymakcalan and Turker (1975) showed that THC causes a concentration-dependent increase in the perfusion pressure of an isolated lung, which could be inhibited by aspirin and by SC19220, a selective antagonist of the prostaglandin EP_1 receptor. Similarly, Wahn *et al.* (2005) showed that AEA increased the perfusion pressure of the rabbit lung, which was blocked by aspirin, nimesulide (COX-2 inhibitor) and an antagonist of the prostanoid EP_1 receptor, but not a thromboxane receptor antagonist. The vasoconstrictor effects of 2-AG in the rat aorta were inhibited by COX-1/2 inhibition and a thromboxane receptor antagonist, and were associated with increases in PGE_2, $PGF_{2\alpha}$ and TXA_2 levels (Stanke-Labesque *et al.*, 2004). Wheal *et al.* (2010) showed that the vasorelaxant response to OEA in first-order branches of the rat mesenteric bed could be enhanced by COX inhibition and by the TXA_2 receptor antagonist vapiprost, suggesting vasoconstrictor prostanoids oppose the vasorelaxant effects of OEA. In human mesenteric arteries, we have also recently observed that the vasorelaxant responses to 2-AG can be enhanced in the presence of the non-selective prostanoid EP and DP antagonist AH6809 (Stanley and O'Sullivan, 2014). Vasodilator prostaglandins are also produced by cannabinoid metabolism in the vasculature. Herradón *et al.* (2007) found that AEA-mediated vasorelaxation of the rat aorta was inhibited by COX-2 inhibition and antagonism of the EP_4 receptor. In the rat pulmonary artery, prostacyclin (IP) receptor antagonism inhibited the vasorelaxant effects of AEA (Baranowska-Kuczko *et al.*, 2012). In human pulmonary arteries, the endocannabinoid virodhamine caused vasorelaxation that was inhibited by the non-selective COX inhibitor indomethacin, which points towards the production of vasorelaxant prostanoids (Kozlowska *et al.*,

2008). Furthermore, in the mesenteric artery, we have shown that 2-AG-induced vasorelaxation can be inhibited using indomethacin and antagonists of the IP or the EP_4 prostanoid receptors (Stanley *et al.*, 2011). Interestingly, cytochrome p450 metabolites of 2-AG cause vasorelaxation, which can at least partially be inhibited by CB_1 antagonism (Awumey *et al.*, 2008).

Alterations in the vascular response to cannabinoids in disease situations

Some studies have now investigated whether the vascular effects of cannabinoids are altered in various disease states. Wheal *et al.* (2007) showed an enhanced vasorelaxant response to AEA in perfused mesenteric beds of rats made hypertensive by chronic NO synthase inhibition. A subsequent study in this model showed this was abolished by capsaicin pretreatment, suggesting an increased TRPV component (Wheal and Randall, 2009). Similarly, O'Sullivan *et al.* (2007) showed enhanced vasorelaxant responses to THC in small resistance mesenteric arteries in the same model of hypertension due to enhanced TRPV and COX involvement. However, in the spontaneously hypertensive rat (SHR) model of hypertension, the vasorelaxant effects of AEA were reduced in the perfused mesenteric bed, and were enhanced in aortic rings (Wheal and Randall, 2009). The enhanced response in SHR aortae was endothelium dependent. Hopps *et al.* (2012) also showed that that vasorelaxant response to oleamide was enhanced in the aorta of SHRs, which could be abolished by capsaicin pretreatment, suggesting enhanced roles for sensory nerves. The COX-1-sensitive component of the response to oleamide was also lost in the SHRs. Taken together, these results suggest that the alterations in the vascular response to cannabinoids in hypertension appear to depend on the artery studied and the model of hypertension.

Domencali and colleagues (2005) showed that the vasorelaxant response to AEA was enhanced in cirrhotic rats and this was associated with an increase in CB_1 and TPRV1 receptor protein (no role for the endothelium). Similarly, Moezi *et al.* (2006) showed that AEA increases mesenteric arteriole diameter in cirrhotic rats, which was blocked by a CB_1 antagonist and was associated with increased CB_1 and TPRV1 receptor protein.

Most recently, it has been shown that the vasorelaxant responses to AEA are reduced in mesenteric arteries from young obese Zucker rats (Lobato *et al.*, 2013). This was associated with decreased CB_1 and CB_2, but not TRPV1, receptor protein expression. We have also shown that the responses to AEA and 2-AG are significantly reduced in the Zucker diabetic model, which appears to be brought about by enhanced metabolism, sometimes to vasoconstrictor products, sensitive to antagonism of the thromboxane receptor (A.J. Wheal *et al.*, unpublished observations).

Together, these studies show that changes in the expression of any of the components bringing about the vascular response to cannabinoids alter their response, with both enhancement and reductions in the vasorelaxant response to endocannabinoids observed. This is more relevant in light of the increasing evidence that plasma concentrations of endocannabinoids in humans are altered in a multitude of disorders including obesity (Bluher *et al.*, 2006), diabetes and insulin resistance (Cote *et al.*, 2007; Abdulnour *et al.*, 2014), obstructive sleep apnea (Engeli *et al.*, 2012), and post-traumatic stress (Hauer *et al.*, 2013), and the impact that these might have on the vasculature.

Conclusions and summary

Cannabinoids cause both vasorelaxation and constriction when applied to vascular preparations. The target sites of action for cannabinoids in the vasculature include CB_1, CB_2, an endothelial-bound cannabinoid receptor (CB_e), TRPV1 (and potentially other TRPVs), GPR55, $5\text{-}HT_{1A}$ and the nuclear receptors PPARα,γ. Potentially as yet uncloned cannabinoid receptor(s) in the vasculature may also exist. The CGRP receptor brings about the vasorelaxation induced through activation of receptors on sensory nerves. Indirect target sites of action for cannabinoids mediated by their metabolic products include the TP and EP_1 prostanoid receptors causing vasoconstriction, and EP_4 and IP receptor causing vasorelaxation. Additionally, cannabinoids modulate the actions of other vasoactive agonists such as acetylcholine, methoxamine, angiotensin II and U46619.

Tables 1 and 2 highlight several gaps in our knowledge on the vascular effects of cannabinoids. To date, most research has focused on AEA, and there is comparatively less known about the vascular effects of other endocannabinoids in different vascular beds. Similarly, only the phytocannabinoid THC has been extensively studied. Discrepancies between results observed between studies may be a result of species differences. Limited human studies to date have revealed both similarities and differences in the vascular effects of cannabinoids and the mechanisms of how relaxation is brought about, but further studies are required to examine different cannabinoids and different vascular beds.

It should be noted that not all cannabinoids have vascular effects. Zygmunt *et al.* (1999) showed that PEA, HU-210 and WIN55,212 do not cause relaxation of rat hepatic and guinea pig basilar arteries. WIN55212-2 (10 μM) failed to produce vasorelaxation of the rabbit aorta, although the response to methanandamide in the same study was sensitive to SR141716A (Mukhopadhyay *et al.*, 2002). Wagner *et al.* (1999) found that WIN55,212, HU-210, THC and 2-AG do not relax the whole mesenteric bed, although these compounds do relax isolated small mesenteric arteries (see Table 1). Methanandamide and Abn-CBD, but not CP55,940 or WIN55212-2, increase NO production in rabbit aortic endothelial cells (McCollum *et al.*, 2007). AEA but not PEA relaxes rat coronary arteries (White *et al.*, 2001). AEA but not methanandamide relaxes the sheep coronary artery (Grainger and Boachie-Ansah, 2001). Neither AEA nor methanandamide evoked a vasodilator response in human small myometrial arteries (Kenny *et al.*, 2002). The mechanisms by which different cannabinoid compounds affect the vasculature differently warrants further research.

Methodological differences may account for some discrepancies in the published literature. The role of the contractile agent used in the vasorelaxation studies may influence study results, for example, Li *et al.* (2010) and

McNeish *et al*. (2012) showed that U46619-induced thromboxane receptor activation alters BK_{ca} channel function, therefore compounds that rely on this pathway in the induction of vasorelaxation may be sensitive to the contractile agent used. Experimental approaches may also influence results. For example, Zygmunt *et al*. (1999; 2002) conducted experiments in the presence of NO and COX inhibitors, and under these conditions, they report that AEA- and THC-induced vasorelaxation in rat hepatic, and rat and guinea pig mesenteric arteries is through TRPV. However, in the absence of NO and COX inhibitors, O'Sullivan *et al*. (2005a) found that vasorelaxation to THC was unaffected by incubation with capsaicin, and that a role for TRPV only became apparent when L-NAME and indomethacin were present.

In conclusion, the range of target sites of actions for cannabinoids in the vasculature is increasing and it is likely that there are still more to be identified. For example, little is known about potential interactions of cannabinoids with adrenoceptors, although a recent study found the phytocannabinoid cannabigerol is a potent α_2-adrenoceptor agonist (Cascio *et al*., 2010). Further work is required to fully understand the physiological consequence of cannabinoid interactions with vascular receptors, and how and why this is altered in pathological situations.

Conflict of interest

None.

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