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REVIEW

Endothelial atypical cannabinoid receptor: do we have enough evidence?

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Cannabinoids and their synthetic analogues affect a broad range of physiological functions, including cardiovascular variables. Although direct evidence is still missing, the relaxation of a vast range of vascular beds induced by cannabinoids is believed to involve a still unidentified non-CB_1, non-CB_2 $G_{i/o}$ protein-coupled receptor located on endothelial cells, the so called endothelial cannabinoid receptor (eCB receptor). Evidence for the presence of an eCB receptor comes mainly from vascular relaxation studies, which commonly employ pertussis toxin as an indicator for GPCR-mediated signalling. In addition, a pharmacological approach is widely used to attribute the relaxation to eCB receptors. Recent findings have indicated a number of GPCR-independent targets for both agonists and antagonists of the presumed eCB receptor, warranting further investigations and cautious interpretation of the vascular relaxation studies. This review will provide a brief historical overview on the proposed novel eCB receptor, drawing attention to the discrepancies between the studies on the pharmacological profile of the eCB receptor and highlighting the $G_{i/o}$ protein-independent actions of the eCB receptor inhibitors widely used as selective compounds. As the eCB receptor represents an attractive pharmacological target for a number of cardiovascular abnormalities, defining its molecular identity and the extent of its regulation of vascular function will have important implications for drug discovery. This review highlights the need to re-evaluate this subject in a thoughtful and rigorous fashion. More studies are needed to differentiate $G_{i/o}$ protein-dependent endothelial cannabinoid signalling from that involving the classical CB_1 and CB_2 receptors as well as its relevance for pathophysiological conditions.

Abbreviations

Abn-CBD, abnormal cannabidiol; ARA-S, N-arachidonyl serine; BK_{Ca}, high-conductance Ca^{2+}-dependent K^+ channels; CB_1 receptor, cannabinoid receptor type 1; CB_2 receptor, cannabinoid receptor type 2; eCB receptor, endothelial cannabinoid receptor; GPR18, GPCR 18; NAGly, N-arachidonyl glycine; O-1918, 1,3-dimethoxy-5-methyl-2-[(1R,6R)-3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-benzene; SR141716, rimonabant; THC, Δ^9-tetrahydrocannabinol; TRP, transient receptor potential

Table of Links

TARGETS	LIGANDS
5-HT receptor	Abn-CBD
α_1 adrenoceptor	Acetylcholine
Akt	Anandamide (AEA)
AT_1 receptor	AM251
BK_{Ca} channels	Apamin
$Ca_V2.2$	Bradykinin
$Ca_V3.1$	Cannabidiol
$Ca_V3.2$	Carbachol
$Ca_V3.3$	Charybdotoxin
CB_1 receptor	Forskolin
CB_2 receptor	HU-210
ERK1/2	Iberiotoxin
Glycine receptors	L-NAME
GPR18	LPI
GPR55	NaGly
GPR119	NO
Ionotropic glutamate receptor	NS1619
IP_3 receptor	O-1602
KCa channels	Oleamide
M_1 muscarinic receptor	Oleoylethanolamide
M_2 muscarinic receptor	Rimonabant (SR141716)
MAPK	Ryanodine
Na^+/Ca^{2+} exchanger (NCX)	THC
Na_V channel	WIN55212-2
Nicotinic acetylcholine receptors	
NOS	
Opioid receptors	
PI3K	
PPARγ	
ROCK	
TRP channels	
TRPV channels	
VEGF receptor	

This Table lists key protein targets and ligands in this document, which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 (Alexander *et al.*, 2013a,b,c,d,f,g).

Introduction

Due to the diverse physiological effects of cannabinoids, the endocannabinoid system has attracted major attention as a potential therapeutic target for a broad range of diseases. While two $G_{i/o}$ protein-coupled cannabinoid receptors, CB_1 and CB_2R receptors, commonly mediate the physiological actions of cannabinoids (Alexander *et al.*, 2013a), their vasodilator effects in a wide range of vascular beds are not thought to involve these classical cannabinoid receptors (Jarai *et al.*, 1999; Wagner *et al.*, 1999; Ho and Hiley, 2003a; Offertaler *et al.*, 2003). A site of action for cannabinoids distinct from CB_1/CB_2 receptors has also been demonstrated in the endothelium of different vascular beds, both in micro- and macrovessels, including the rat (Milman *et al.*, 2006; Herradon *et al.*, 2007; Lopez-Miranda *et al.*, 2010) and rabbit aorta (Mukhopadhyay *et al.*, 2002; McCollum *et al.*, 2007), rat (Baranowska-Kuczko *et al.*, 2012) and human pulmonary artery (Kozlowska *et al.*, 2007). The molecular mechanisms, by which cannabinoids produce vasodilatation, have not

been fully elucidated, but are believed to involve still unidentified CB receptors located on endothelial cells, so called endothelial cannabinoid receptors (eCB receptors).

Identification of the eCB receptor and its particualr signalling cascade is not merely a pure theoretical challenge. The selection of compounds with reduced psychoactivity has emerged as a promising therapeutic strategy for a vast number of diseases. Much interest in the identification of the eCB receptor and its pharmacological characterization stems from its promising therapeutic potential in a large number of disorders (Robson, 2013), especially considering its lack of psychiatric side effects, which result from stimulation of central CB_1 receptors. Identification of the mechanisms of the CB_1/CB_2 receptor-independent actions of cannabinoids in the cardiovascular system is fuelled by discoveries of antioxidant, anti-inflammatory (Booz, 2011) and cardioprotective properties of cannabinoids that occur independently of CB_1/CB_2 receptors (Fouad *et al.*, 2013). Accordingly, considerable effort is being assigned to identify the signalling mechanisms of eCB receptor-attributed effects, as selective eCB receptor targeting is thought to have great therapeutic potential.

Although 15 years have passed since the first publication of indications of the novel eCB receptor (Jarai *et al.*, 1999; Wagner *et al.*, 1999), its molecular identity is still unclear. Moreover, it is not yet conclusive that vascular effects commonly attributed to eCB receptor actually require G-protein coupled receptor (GPCR). The weak link is that our current knowledge on the eCB receptor, its pharmacology and signalling profile almost exclusively relies on the interpretation of data obtained in numerous arterial relaxation studies. These studies (i) commonly use a $G_{i/o}$ protein inhibitor, pertussis toxin, to demonstrate the involvement of GPCRs (Herradon *et al.*, 2007; Hoi *et al.*, 2007; Kozlowska *et al.*, 2007; O'Sullivan *et al.*, 2009; Parmar and Ho, 2010; Alsuleimani and Hiley, 2013) and (ii) presume the eCB receptor agonists and antagonists used are selective. The sensitivity of the vasodilatation to pertussis toxin, the phytocannabinoid cannabidiol, its analogue O-1918 and the CB_1 receptor antagonist rimonabant (SR141716A) became classic tools to propose and in further studies support the presence of *non-CB_1/non-CB_2* endothelial G protein-coupled CB receptors referred to in the literature as 'abnormal cannabidiol', 'endothelial anandamide' or 'atypical endothelial cannabinoid receptor' (Jarai *et al.*, 1999; Wagner *et al.*, 1999; Mukhopadhyay *et al.*, 2002; Herradon *et al.*, 2007; Baranowska-Kuczko *et al.*, 2012). Due to the abundant pharmacological effects of cannabinoids and cannabinoid-like compounds outlined by Alexander and Kendall (2007), possible interactions of these compounds with unspecified CB receptor-independent targets will not always be detected in relaxation studies. Vascular responsiveness to cannabinoids varies not only between various vascular beds/species, but also even within the same vascular bed. For example, in the rat isolated aorta, the maximal relaxation to anandamide varies from 22 (O'Sullivan *et al.*, 2005b) to 60% (Milman *et al.*, 2006) of the imposed contraction and may (Herradon *et al.*, 2007) or may not depend (O'Sullivan *et al.*, 2005b) on the presence of the endothelium. Similarly, in rat small mesenteric arteries, the relaxation to anandamide was shown to be either endothelium-dependent (O'Sullivan *et al.*, 2004) or endothelium-independent (White and Hiley, 1997). A critical look at studies published reveals a number of further inconsistencies in the mechanisms of vasodilatation commonly attributed to eCB receptors, including the sensitivity to CB_1 receptor antagonists rimonabant (Jarai *et al.*, 1999; Harris *et al.*, 2002; Ho and Hiley, 2003a; Milman *et al.*, 2006) and AM251 (Ho and Hiley, 2003a; Hoi *et al.*, 2007), NO synthase inhibitors (Jarai *et al.*, 1999; Harris *et al.*, 2002; Ho and Hiley, 2003a; Kozlowska *et al.*, 2007; McCollum *et al.*, 2007) and gap junction inhibitors (Brandes *et al.*, 2002; Harris *et al.*, 2002; Randall *et al.*, 2002; Ho and Hiley, 2003a). Thus, anandamide was reported either to promote (Chaytor *et al.*, 1999; Randall *et al.*, 2002) or inhibit (Fleming *et al.*, 1999; Brandes *et al.*, 2002) gap junction communications, while rimonabant has an inhibitory effect (Chaytor *et al.*, 1999). An ability of cannabinoids to affect gap junctions, a critical player in vascular electrical and mechanical responses, further emphasizes certain restrictions when using wire myography as the only approach to characterize the mechanisms of action of cannabinoids on endothelial cells and vascular pharmacology of CB receptor ligands. Table 1 summarizes the key findings on the pharmacology of relaxant responses to cannabinoids, highlighting substantial disparities in the pharmacological profiles of these responses.

This paper represents a critical overview of the relevant publications, drawing particular attention to certain discrepancies in the field. Numerous GPCR-independent actions of the compounds widely used as selective agonists and antagonists of the novel eCB receptor highlight the need for a re-evaluation of the subject in a thoughtful and rigorous fashion.

CB receptor-independent effect of rimonabant

The laboratory of G. Kunos was the first to propose the novel CB receptor in the vascular endothelium in 1999 (Jarai *et al.*, 1999; Wagner *et al.*, 1999). In the rat isolated perfused mesenteric arterial bed precontracted with phenylephrine, a bolus injection of anandamide was shown to produce a sustained and pronounced concentration-dependent vasodilatation (Wagner *et al.*, 1999), which had an endothelium-dependent component sensitive to 1 μM of the CB_1 receptor antagonist rimonabant. The receptor was suggested to be distinct from the CB_1 receptor as the CB_1 receptor agonists WIN55212-2 and HU-210 failed to reproduce the vasodilatation. These observations led the authors to conclude that the anandamide-induced mesenteric vasodilatation is mediated by a novel anandamide receptor located on endothelial cells. Consistent with this notion, abnormal cannabidiol (Abn-CBD), a synthetic analogue of the non-psychoactive phytocannabinoid cannabidiol, was shown to produce rimonabant-sensitive hypotension and endothelium-dependent mesenteric vasodilatation independently of NO (Jarai *et al.*, 1999). The response was also preserved in mice lacking either CB_1 receptors or both CB_1 and CB_2 receptors and, hence, appeared to depend on molecular target(s) distinct from CB_1 and CB_2 receptors. Based on these findings, it was concluded that Abn-CBD is a selective agonist, while cannabidiol is a selective antagonist of novel anandamide receptor present in endothelial cells (Jarai *et al.*, 1999).

Table 1

Summary of pharmacology of CB_1/CB_2 receptor-independent relaxant responses to cannabinoids

Ligands	SR141716A	AM-251	O-1918	L-NAME	De-endothelization	PTX	FAAH inhibitors	APA + ChTx	IbTx	TRPV1 antagonism	αGA	pEC_{50} (μM)	Rmax, %	Vascular bed	Reference
AEA	Inhibition			No effect	No effect	Inhibition		No effect	No effect			6.3	95	RSMA	White and Hiley, 1997
AEA	Inhibition			No effect	Slight inhibition					Slight inhibition	Inhibition	6.2	94	RMA	Harris *et al.*, 2002
AEA	Inhibition	Inhibition	Inhibition	No effect	Inhibition			Inhibition		Inhibition	Inhibition	5.7	89	RSMA	O'Sullivan *et al.*, 2004
AEA	Inhibition	Inhibition	No effect	No effect	No effect			No effect		Inhibition	No effect	5.0	33	RMA	O'Sullivan *et al.*, 2004
AEA									No effect				80	RMA	Yang *et al.*, 2007
AEA	Inhibition	No effect			No effect		No effect		Inhibition	No effect	No effect	6.8	45	RCA	White *et al.*, 2001
AEA										Inhibition		6.7	97	RSMA	White *et al.*, 2001
AEA	No effect			Inhibition	Inhibition	Inhibition	Inhibition	No effect		No effect		5.9	51	RA	Herradon *et al.*, 2007
AEA	No effect	No effect			No effect	Inhibition				No effect		5.9	22	RA	O'Sullivan *et al.*, 2005b
AEA		No effect										5.2	90	HPA	Kozlowska *et al.*, 2007
AEA		No effect	Inhibition	Inhibition	Inhibition		Inhibition	Inhibition		No effect		5	90	RPA	Baranowska-Kuczko *et al.*, 2012
methAEA	Inhibition				Inhibition	Inhibition				Slight inhibition	No effect	0.6	80	RabA	Mukhopadhyay *et al.*, 2002
Abn-CBD	Inhibition			No effect	Inhibition			Inhibition	No effect	No effect				RPIMAB	Jarai *et al.*, 1999
Abn-CBD			Inhibition									3.0	107	RMA	Johns *et al.*, 2007
Abn-CBD			Inhibition	No effect		Inhibition		Inhibition		No effect		6.7	93	RMA	Offertaler *et al.*, 2003
Abn-CBD	Inhibition	Inhibition		No effect		No effect		Inhibition	No effect		No effect	6.2	93	RSMA	Ho and Hiley, 2003a
Abn-CBD		No effect	Inhibition	Inhibition	Inhibition		Inhibition	Inhibition		No effect		4.6	82	RPA	Baranowska-Kuczko *et al.*, 2012
Abn-CBD			Inhibition			no effect						2		RMA	Milman *et al.*, 2006
abn-cnd			Inhibition	No effect	Inhibition	Inhibition		Inhibition				4.8	110	HPA	Kozlowska *et al.*, 2007
NAGly		No effect	Inhibition	Inhibition	Inhibition	Inhibition	No effect	Inhibition	Inhibition	No effect		5.8	88	RSMA	Parmar and Ho, 2010
ARA-S				Inhibition					Inhibition			4.9	84	RSMA	Parmar and Ho, 2010
ARA-S			Inhibition			No effect						0.55	95	RMA	Milman *et al.*, 2006
ARA-S	No effect		No effect			Inhibition						1.2	60	RA	Milman *et al.*, 2006
Oleamide	Inhibition	No effect	Inhibition	Inhibition	Inhibition	Inhibition		Inhibition		Inhibition		1.2	99	RSMA	Hoi and Hiley, 2006

Abn-CBD, abnormal cannabidiol; AEA, anandamide; APA + ChTx, apamin plus charybdotoxin; FAAH, fatty acid amide hydrolase; HPA, human pulmonary artery; IbTx, iberiotoxin; L-NAME, NG-Nitro-L-arginine methyl ester; RA, rat aorta; RCA, rat coronary artery; RPA, rat pulmonary artery; RSMA, rat small mesenteric artery; TRPV, transient receptor potential cation channel subfamily V.

Table 2

Cannabinoid receptor-independent molecular targets for the putative eCB receptor antagonists

Putative antagonists of eCB receptor	Targets or CB receptor-independent effects	Reference
SR141716A	Antagonizes endothelium-dependent relaxation to carbachol, bradykinin, ionomycin and A23187	Wagner *et al.*, 1999 Randall *et al.*, 1996 White and Hiley, 1997
SR141716A	Antagonizes endothelium-independent relaxation to anandamide	Randall *et al.*, 1996 Harris *et al.*, 2002 Ho and Hiley, 2003a
SR141716A	Inhibition of myoendothelial communications	Chaytor *et al.*, 1999
SR141716A	Inhibits unidentified K^+ channels	Bukoski *et al.*, 2002
SR141716A	Inhibits relaxation induced by Ca^{2+}-re-addition	Bukoski *et al.*, 2002
O-1918	Inhibits relaxation of denuded arteries to sodium nitroprusside Inhibits BK_{Ca} channels Inhibits Na^+-Ca^{2+} exchanger	Parmar and Ho, 2010 Godlewski *et al.*, 2009 Bondarenko *et al.*, 2013
Cannabidiol	Acts as PPAR γ ligand Antagonist of α_1 adrenoceptors Inhibits voltage-gated Na^+ channels Inhibits 5-HT_3 receptors Activates TRPV channels	O'Sullivan *et al.*, 2009 Pertwee *et al.*, 2002 Hill *et al.*, 2014 Yang *et al.*, 2010 Hassan *et al.*, 2014

BK_{Ca}, high-conductance Ca^{2+}-dependent K^+ channels; SR141716, rimonabant.

It is interesting to note that the authors also observed an antagonistic effect of 1 μM rimonabant on endothelium-dependent relaxation elicited by administration of a low concentrations of the Ca^{2+} ionophore ionomycin (Wagner *et al.*, 1999). In isolated vessels, ionomycin evokes endothelial cell hyperpolarization, similar to that produced by acetylcholine (Bondarenko and Sagach, 2006). The antagonistic effect of 1 μM rimonabant on endothelium-dependent relaxation induced by the Ca^{2+} ionophore A23187, carbachol and bradykinin was also demonstrated by others (Randall *et al.*, 1996; Randall and Kendall, 1997; White and Hiley, 1997), indicating that rimonabant may act through targets other than the CB_1 receptor and putative eCB receptor. However, this possibility was discarded by the authors (Jarai *et al.*, 1999) because of a reported failure of 1 μM rimonabant to inhibit the relaxation to NS1619, an opener of high-conductance Ca^{2+}-dependent K^+ channels (BK_{Ca} channels) (White and Hiley, 1998). At the same time, the contribution of BK_{Ca} channels to the Abn-CBD-evoked mesenteric vasodilatation was not confirmed because of its resistance to a combination of apamin plus iberiotoxin (Jarai *et al.*, 1999). The inhibitory effect of 1 μM rimonabant on the relaxation induced by ionomycin was attributed to the stimulated release of anandamide from the endothelium in the presence of ionomycin (Wagner *et al.*, 1999).

Of interest, earlier studies performed on the rat mesentery showed that the anandamide-induced vasodilatation is unaffected by endothelial cell denudation (Randall *et al.*, 1996; White and Hiley, 1997) and NOS blockade (White and Hiley, 1997). Nevertheless, even in endothelium-denuded vessels, the response was antagonized by 0.1–1 μM rimonabant (Randall *et al.*, 1996; Harris *et al.*, 2002; Ho and Hiley, 2003b; O'Sullivan *et al.*, 2004), strongly indicating a non-endothelial site of action of the blocker. Rimonabant was also shown to inhibit the relaxation of mesenteric arteries induced by the re-addition of Ca^{2+} (Bukoski *et al.*, 2002). A similar blocking effect was observed for O-1918. The effect was detected in arteries from both control and CB_1 receptor-deficient mice, excluding the effect of rimonabant on CB_1 receptors. Additionally, in patch-clamp experiments, rimonabant inhibits an unidentified macroscopic K^+ current other than that attributable to BK_{Ca} channels (Bukoski *et al.*, 2002). In another early study (Chaytor *et al.*, 1999), anandamide was shown to relax isolated rings of rabbit superior mesenteric artery through an endothelium-dependent and endothelium-independent mechanism. In this study, rimonabant at 10 μM attenuated endothelium-dependent, but not endothelium-independent, relaxation through inhibition of myoendothelial communications. Altogether, these data clearly indicate that rimonabant exhibits a number of effects independently of CB receptors and, hence, further work is needed to identify whether rimonabant-mediated suppression of eCB receptor-attributable vasodilatation is mediated via CB receptor-independent mechanisms. Pharmacological targets of putative antagonists of eCB receptors and their CB receptor-independent effect on vasodilaation are summarized in Table 2.

CB receptor-independent effect of O-1918

In 2003, the group of G. Kunos further substantiated the concept of the involvement of novel eCB receptor in the vasorelaxation induced by endocannabinoids by showing

that in endothelium-intact, but not endothelium-denuded, rat mesenteric arterial segments, the cannabidiol analogue O-1918 concentration-dependently inhibits the relaxation to Abn-CBD, a ligand for the putative eCB receptor (Offertaler *et al.*, 2003). These observations allowed the authors to propose O-1918 as a selective antagonist of the Abn-CBD receptor located on endothelial cells. Since then, numerous vascular relaxation studies replicated on different vascular beds employed O-1918 as a selective antagonist of eCB receptors (Hoi *et al.*, 2007; Kozlowska *et al.*, 2007; Zakrzeska *et al.*, 2010; Baranowska-Kuczko *et al.*, 2012; Caldwell *et al.*, 2013), offering further support for existence of novel endothelially located CB receptors. However, in de-endothelized and permeabilized rabbit pulmonary artery strips, Abn-CBD still produced vasodilatation, the response was prevented by O-1918, and only partially reduced by AM251, SR141716A and pertussis toxin (Su and Vo, 2007), indicating a GPCR-independent action of Abn-CBD and O-1918 on molecular targets located on smooth muscle cells. Recently, GPCR-independent effects of O-1918 on distinct molecular targets located in the vasculature have been demonstrated (Godlewski *et al.*, 2009; Bondarenko *et al.*, 2013), indicating the possibility that the inhibitory action of O-1918 on vasodilatation is mediated by a non-specific effect. Consistent with this, at concentrations used to verify the role of eCB receptors (3–10 μM), O-1918 was also shown to significantly attenuate the relaxant responses of endothelium-denuded mesenteric arteries to sodium nitroprusside, a NO donor (Parmar and Ho, 2010). This observation suggests that O-1918 has CB receptor-independent target(s) located on smooth muscle cells. Indeed, in a concentration range used for inhibition of eCB receptor-attributable relaxation, O-1918 inhibits the activity of BK_{Ca} channels (Godlewski *et al.*, 2009) and the Na^+-Ca^{2+} exchanger (Bondarenko *et al.*, 2013), key players regulating vascular contractility. These effects do not require GPCR, excluding O-1918 as a selective antagonist of eCB receptors and indicating that the interpretation of results where O-1918 has been assumed to be a selective eCB receptor antagonist should be treated with caution. The conclusions of authors on involvement of eCB receptors in relaxation are frequently based on the supposed selectivity of eCB receptor agonists and antagonists, while additional off-target effects of the compounds are not critically considered. For example, in a recent study performed on rat pulmonary artery, a vascular bed of endothelial cells expressing functional BK_{Ca} channels (Vang *et al.*, 2010), the putative eCB receptor antagonist O-1918, which acts as a BK_{Ca} channel inhibitor (Godlewski *et al.*, 2009), was shown to attenuate the anandamide-evoked relaxation (Baranowska-Kuczko *et al.*, 2012). Conclusively, it is a general recommendation that the site of action of O-1918, a commonly used eCB receptor inhibitor claimed to be selective, needs to be unambiguously demonstrated.

CB receptor-independent effect of cannabidiol

Cannabidiol has a relatively low affinity for classical cannabinoid receptors, and is commonly used as an antagonist of the novel CB receptor. However, the relevant experimental data is also controversial and indicates the presence of multiple target sites for cannabidiol. Thus, unlike in rat mesentery (Jarai *et al.*, 1999) and rat (Baranowska-Kuczko *et al.*, 2012) and human (Kozlowska *et al.*, 2007) pulmonary artery, where cannabidiol antagonizes the eCB receptor-attributed relaxation, in rat aorta, cannabidiol actually produces vasodilatation (O'Sullivan *et al.*, 2009) by acting as a PPAR ligand. In contrast to findings of the earlier study (Jarai *et al.*, 1999), cannabidiol was reported to produce relaxation in rat isolated mesenteric arteries (Offertaler *et al.*, 2003), suggesting that this compound may act both as a silent antagonist and a partial agonist of eCB receptors. At 5–10 μM, concentrations commonly used to antagonize the eCB receptor-attributed vasodilatation, cannabidiol attenuates contractile responses to agonists of α_1 adrenoceptors, as demonstrated in mouse isolated vas deferens (Pertwee *et al.*, 2002). In addition, cannabidiol was shown to inhibit Na_v channels (Hill *et al.*, 2014), $5\text{-}HT_{3A}$ receptor-mediated currents (Yang *et al.*, 2010), and via activation of one or more transient receptor potential cation channel subfamily V (TRPV), enhance phagocytosis of mouse microglial BV-2 cells (Hassan *et al.*, 2014).

K_{Ca} channels in the eCBR-attributable relaxation

The identity of the K^+ channel subtypes involved in the relaxation to cannabinoids is still controversial. In rat isolated small mesenteric artery, the relaxation to N-arachidonylglycine (NAGly) is sensitive to iberiotoxin, a blocker of Ca^{2+}-activated K^+ (K_{Ca}) channels of large (BK_{Ca}) conductance, and has endothelium-dependent component (Parmar and Ho, 2010). In contrast, the relaxation to anandamide was reported to be endothelium-independent and insensitive to iberiotoxin either alone or in combination with apamin (White and Hiley, 1997). In rat isolated mesenteric arteries, charybdotoxin, the BK_{Ca} and IK_{Ca} channel blocker, inhibited the relaxation to Abn-CBD (Offertaler *et al.*, 2003). The effect was slightly potentiated by the additional presence of apamin, while apamin alone had no effect. The combination of charybdotoxin plus apamin was effective at inhibiting the eCB receptor-attributed vasodilator responses in a number of other vascular beds, including human (Kozlowska *et al.*, 2007) and rat (Baranowska-Kuczko *et al.*, 2012) pulmonary artery, implicating K_{Ca} channels of small (SK_{Ca}), intermediate (IK_{Ca}) and large conductance. In rat coronary arteries, the relaxation to anandamide was shown to be endothelium-independent and sensitive to iberiotoxin (White *et al.*, 2001). Interestingly, the endothelial cells of rat aorta express functional IK_{Ca} and SK_{Ca} channels (Marchenko and Sage, 1996; Bondarenko *et al.*, 2012), and anandamide-induced aortic relaxation is insensitive to a combination of apamin and charybdotoxin (Herradon *et al.*, 2007).

The role of NO in relaxation to cannabinoids

In rat isolated small mesenteric artery, the relaxation attributable to novel CB receptors was reported to be either

sensitive (Hiley and Hoi, 2007; Parmar and Ho, 2010) or insensitive to NOS inhibition (Ho and Hiley, 2003a; 2004; O'Sullivan *et al.*, 2004). In macrovessels, such as rat (Herradon *et al.*, 2007) and rabbit (Mukhopadhyay *et al.*, 2002) aorta and rat pulmonary artery (Baranowska-Kuczko *et al.*, 2012), the CB_1/CB_2 receptor-independent endothelium-dependent relaxation is diminished or fully inhibited by inhibition of NOS and was shown to be accompanied by an increased NO release (McCollum *et al.*, 2007; Baranowska-Kuczko *et al.*, 2012). Conversely, in human pulmonary artery, the relaxation was reported to be insensitive to NOS inhibition (Kozlowska *et al.*, 2007). The reasons for these discrepancies are not clear but probably represent regional/species differences.

Is GPCR 55 (GPR55) a novel CB receptor?

In addition to CB_1 and CB_2 receptors, GPR55 has been described as a target for cannabinoid ligands (Ryberg *et al.*, 2007; Pertwee, 2010; Liu *et al.*, 2014). Initially, GPR55, which, in addition to classical cannabinoids and cannabinoid-like compounds [Abn-CBD, O-1602, N-arachidonyl serine (ARA-S)], is activated by lysophosphatidylinositol (LPI), an endogenous lysophospholipid not related to CB receptor ligands, has been proposed to represent the eCB receptor (Baker *et al.*, 2006). Activation of GPR55 by several ligands was shown to increase intracellular Ca^{2+} in several cell types (Kohno *et al.*, 2006; Lauckner *et al.*, 2008), including endothelial cell line EA.hy926 (Waldeck-Weiermair *et al.*, 2008), where GPR55 stimulation by LPI produces transient hyperpolarization (Bondarenko *et al.*, 2010), suggesting the functional importance of GPR55 in vascular physiology. In human dermal microvascular endothelial cells, GPR55 knockdown partially inhibited ARA-S-induced migration and activation of Akt and ERK1/2 (Zhang *et al.*, 2010). Additionally, in GPR55-knockdown cells, the ARA-S-induced VEGF production was significantly decreased as compared with the control GPR55-expressing cells (Zhang *et al.*, 2010). However, the BP lowering effect and the vasodilator responses of isolated mesenteric arteries to O-1602 and Abn-CBD appeared to be unaltered in GPR55-deficient mice (Johns *et al.*, 2007). These results indicate that although current knowledge implicates GPR55 in cell migration and growth, GPR55 is not critically involved in the vasodilatation to O-1602 and abn-cbd, and accordingly, GPR55, perhaps, is unlikely to represent the molecular identity of the eCB receptor. An additional set of observations at first glance arguing against GPR55 as the eCB receptor is a discrepancy between the reported $G_{i/o}$ protein requirement for the vasodilator responses (Mukhopadhyay *et al.*, 2002; Offertaler *et al.*, 2003) and non-$G_{i/o}$ protein-mediated GPR55 signalling cascade reported in a number of studies (Lauckner *et al.*, 2008; Henstridge *et al.*, 2009; Brown *et al.*, 2011). However, given the uncertainties over the requirement of $G_{i/o}$ proteins in the eCB receptor-attributed vasodilatation discussed further below and the ability of shared GPR55/GPR18 signalling through G_q protein to increase intracellular Ca^{2+} (Lauckner *et al.*, 2008; Console-Bram *et al.*, 2014), the association between GPR55 and the eCB receptor should still be considered.

Is GPCR 18 (GPR18) a novel CB receptor?

Another recently emerged candidate for the eCB receptor is GPR18 activated by Δ^9-tetrahydrocannabinol (THC), anandamide, Abn-CBD and the anandamide metabolite NAGly (Kohno *et al.*, 2006; McHugh *et al.*, 2012; Takenouchi *et al.*, 2012). Stimulation of GPR18 by several ligands increases intracellular Ca^{2+} in several cell types (Kohno *et al.*, 2006; Console-Bram *et al.*, 2014), suggesting that vascular GPR18-dependent signalling may have functional implications. In microglial cells, at picomolar and low nanomolar concentrations range, NAGly and Abn-CBD drive cell migration in a GPR18-dependent manner (McHugh *et al.*, 2010). Pertussis toxin was found to attenuate the Abn-CBD- and NAGly-induced cell migration (Mo *et al.*, 2004; McHugh *et al.*, 2010). Additionally, the toxin antagonized the NAGly-induced inhibition of forskolin-stimulated cAMP production (Kohno *et al.*, 2006) and activation of MAPK by Abn-CBD (Offertaler *et al.*, 2003), anandamide and NAGly (McHugh *et al.*, 2010; 2012), suggesting that the effect is mediated by $G_{i/o}$ protein.

Based on observations made on microglial cell migration, GPR18 was proposed to be a molecular candidate for the Abn-CBD receptor (McHugh *et al.*, 2010). Notably, both GPR18 mRNA and protein have recently been identified in isolated arteries and endothelial cells (Ho and Yeung, 2009; MacIntyre *et al.*, 2014; Wilhelmsen *et al.*, 2014). However, a correlation between the GPR18 level and the dilator responses is missing and, hence, the role of GPR18 in vasoactivity and other vascular functions is still unclear. There is also inconsistency in the effects of GPR18 ligands on endothelium-dependency of the dilator responses even within the same vascular bed. Thus, in rat isolated small mesenteric arteries, the relaxation to GPR18 agonists anandamide (O'Sullivan *et al.*, 2004), NAGly (Parmar and Ho, 2010) and Abn-CBD (Ho and Hiley, 2003a) has a strong endothelium-dependent component, while the dilator responses of the same arterial bed to THC (O'Sullivan *et al.*, 2005a) and, curiously, anandamide (White and Hiley, 1997; Ho and Hiley, 2003b) were shown to be endothelium-independent.

The stimulating effects of NAGly and Abn-CBD on cell migration were insensitive to rimonabant, but antagonized by O-1918 and ARA-S, a structurally related to anandamide non-psychoactive endocannabinoid with weak affinity for GPR18 (McHugh *et al.*, 2010). However, in rat isolated aorta and mesenteric artery, ARA-S produces a significant endothelium-dependent vasodilatation with potency similar to that evoked by Abn-CBD (Milman *et al.*, 2006), an observation which is not consistent with the ARA-S being a GPR18/Abn-CBD receptor antagonist. Within the same concentration range, ARA-S directly stimulates the BK_{Ca} channel (Godlewski *et al.*, 2009). Unlike in the mesentery, the aortic relaxation to ARA-S was blocked by pertussis toxin, suggesting the involvement of a GPCR other than GPR18 in aortic, but not mesenteric, vasodilatation. Oleamide, an endogenous lipid that is structurally related to anandamide, produces vasorelaxation with an endothelium-dependent component in rat isolated small mesenteric arteries (Hoi and Hiley, 2006). Consistent with the pharmacological profile of eCB receptors,

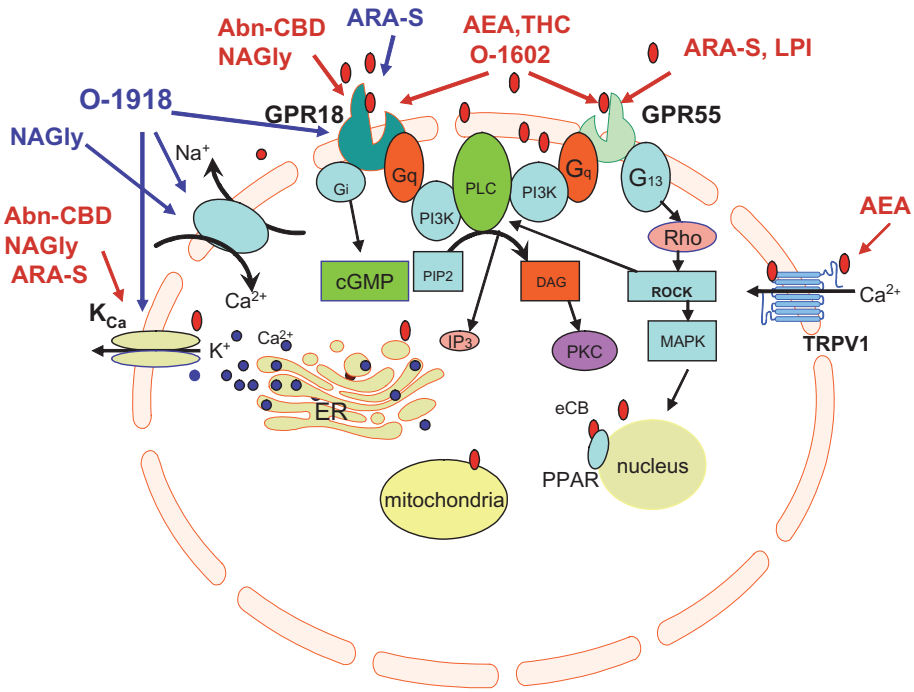

Figure 1

Schematic illustration of current status of research of CB_1/CB_2 receptor-independent endothelial cannabinoid signalling. Red arrows indicate stimulating effects, blue arrows indicate inhibitory effects.

the relaxation was sensitive to rimonabant, O-1918 and pertussis toxin pretreatment. There are, unfortunately, no reports that oleamide binds GPR18 and, hence, it is difficult to assign the mechanism of relaxation to GPR18 or another putative GPCR. The identity of eCB receptor as GPR18 was further questioned by the demonstration that oleoylethanolamide, the anandamide-like monounsaturated fatty acid, which was shown to be a GPR119, but not a GPR18 agonist (Overton *et al.*, 2008), produces O-1918-sensitive endothelium- and NO-dependent relaxation in third branches of rat superior mesenteric artery (Alsuleimani and Hiley, 2013). The recent demonstration of an inability of the GPR55/GPR18 agonist O-1602 to mimic the effects of Abn-CBD and NAGly in ocular signalling system (Caldwell *et al.*, 2013; MacIntyre *et al.*, 2014) casts further doubts on the selectivity of the compounds involved, further challenging the concept that the effects of NAGly and Abn-CBD are solely mediated by GPR18. Given the discrepancy between the levels of GPR18 expression and NAGly in different tissues (Alexander, 2012), a link between NAGly and GPR18 and the identification of GPR18 as the Abn-CBD receptor, the stimulation of which causes vasodilation, seems not so straightforward. Activation of GPR18 by NAGly was not established in a study employing high-throughput β-arrestin-based screening (Yin *et al.*, 2009). The application of NAGly to GPR18-expressing rat sympathetic neurons did not inhibit N-type ($Ca_v2.2$) Ca^{2+} currents, a primary downstream effector of $G_{i/o}$ proteins, but instead potentiated the currents (Lu *et al.*, 2012). Recent findings indicate that GPR18 is coupled to several effector pathways through both $G_{i/o}$ and $G\alpha_q$ proteins (Console-Bram *et al.*, 2014). Signalling via a specific pathway is likely to be context-specific. Signalling via a single receptor coupled to different G-protein subunits may partially explain the controversies in eCB receptor pharmacology. Nevertheless, the association between GPR18 and the eCB receptor-attributed signalling is not yet proven. A schematic figure explaining the current status of the possible existence and signalling through eCB receptors is shown in Figure 1.

Limits of cell culture models

An additional caveat in vascular cannabinoid research is the common use of cell culture models, a condition that may profoundly affect the signalling profile, not reflecting the situation *in situ* but rather a diseased state. Many aspects of endothelial cell signalling, such as functional expression of ion channels/receptors, change dramatically in culture conditions (Sandow and Grayson, 2009). It is well known that endothelial cells grown in culture cease to produce mRNA for M_1 and M_3 muscarinic receptors and, consequently, cultured endothelial cells are unresponsive to acetylcholine (Marchenko and Sage, 1993). Cell culture-associated abnormalities in endothelial cell signalling are also associated with TRPV3 channels and ryanodine-sensitive stores (Bondarenko and Sagach, 1997; Kohler *et al.*, 2001). In addition, the alterations are undoubtedly relevant for endocannabinoid signalling mechanisms. Notably, while some reports utilizing the human endothelial cell line EA.hy926 indicate the expression of the CB_1 receptor and its involvement in the mobilization of intracellular Ca^{2+} in response to anandamide (Waldeck-Weiermair *et al.*, 2008), other studies failed to detect CB_1

receptor expression in the same cell line (Liu *et al.*, 2000). A further potential limitation is associated with the studying of GPCR signalling pathways in heterologous expression systems. It is worth noting that in transfected cells, recombinant opioid and CB receptors operate through the same pool of G proteins, while in cells endogenously co-expressing CB_1 with opioid receptors, the signalling occurs through distinct pools of G proteins (Shapira *et al.*, 2000).

An additional caveat associated with utilizing heterologous expression systems is an essential dependency of CB_1 receptor- and GPR55-mediated cannabinoid signalling on the lipid composition of the plasma membrane (Gasperi *et al.*, 2012). Keeping in mind that cannabinoids target distinct GPCR-independent ion transport systems, the functional activity of which is regulated by membrane cholesterol, cell culture models may not be able to represent the 'native' environment and, most likely, the evoked response is context-dependent. Conclusively, studying cannabinoid signalling in a non-native environment may not reflect a true picture and complicates data interpretation, indicating a need for alternative, more physiological, methodological approaches.

GPCR-independent targets of CB receptor ligands

The mechanism of action of endocannabinoids is not limited to GPCRs. Recently, a growing number of studies have convincingly shown that, at physiologically relevant concentrations, in addition to GPCR-dependent effects, bioactive lipids affect the properties of various ion transport mechanisms independent of GPCRs. The targets affected include voltage- and ligand-gated ion channels, namely the nicotinic acetylcholine, 5-HT and glycine receptors and ionotropic glutamate receptors (Oz, 2006; Alexander and Kendall, 2007; Barana *et al.*, 2010). Anandamide in the concentration range of 1–10 μM causes a significant inhibition of voltage-gated Na^+ channels in mouse cortical (Nicholson *et al.*, 2003) and rat dorsal root ganglion neurons (Nicholson *et al.*, 2003; Kim *et al.*, 2005). Various lipoamino acids, including NAGly, were recently shown to inhibit $Ca_v3.1$, $Ca_v3.2$ and $Ca_v3.3$ channels (Ross *et al.*, 2009) and currents mediated by recombinant $GABA_A$ receptors (Baur *et al.*, 2013). NAGly enhances the inhibitory glycinergic synaptic transmission by blocking glycine uptake (Jeong *et al.*, 2010).

While much is known about the GPCR-independent effects of endocannabinoids and bioactive lipids on ion transport mechanisms in the nervous system, the vascular molecular targets are much less explored. In recent years, evidence has accumulated suggesting that endocannabinoids and lipid compounds exert their effect on endothelial cells by targeting both GPCR-dependent and GPCR-independent targets, including non-selective transient receptor potential (TRP) channels (Bondarenko *et al.*, 2010), Na^+-K^+-ATPase (Bondarenko *et al.*, 2010) and different K_{Ca} channels (Bondarenko *et al.*, 2011a,b). In a GPCR-independent manner, anandamide and NAGly inhibit both forward and reverse modes of Na^+-Ca^{2+} exchanger in endothelial cells (Bondarenko *et al.*, 2013). In addition, NAGly was shown to directly potentiate the BK_{Ca} channels (Bondarenko *et al.*, 2013) and inhibit store-operated Ca^{2+} entry in different cell types (Deak *et al.*, 2013). Because vascular signalling and function are chiefly regulated by Na^+-Ca^{2+} exchanger (Bondarenko, 2004; Andrikopoulos *et al.*, 2011), IK_{Ca}, BK_{Ca} (Vang *et al.*, 2010; Bondarenko *et al.*, 2012; Wulff and Kohler, 2013) and different members of TRP channels, identification of their contribution to GPCR-dependent and -independent cannabinoid signalling still needs to be clarified.

There is also a certain disparity in EC_{50} values of some endocannabinoids as GPR18 ligands and the EC_{50} values of the evoked relaxation. The original study of Kohno *et al.* (2006), who first identified NAGly as a ligand of GPR18, showed that this lipoamino acid concentration-dependently inhibits cAMP formation in a pertussis toxin-sensitive manner, with a calculated IC_{50} value of 20 nM. However, in rat small mesenteric arteries, NAGly evoked a concentration-dependent relaxation with an EC_{50} value of 5.8 μM (Parmar and Ho, 2010), which is several orders higher than that required to activate GPR18. At these concentrations, NAGly exhibits a direct GPCR-independent effect on a number of ion transport systems expressed in both endothelial and smooth muscle cells (Bondarenko *et al.*, 2013; Deak *et al.*, 2013). In endometrial cell line, HEC-1B, THC was shown to be a full agonist for GPR18, with a calculated EC_{50} value of 0.96 μM (McHugh *et al.*, 2012). However, the THC-induced relaxation occurred at higher concentrations, with an EC_{50} value of 5.8 μM (O'Sullivan *et al.*, 2005b). Similar observations were made with another GPR18 agonist, Abn-CBD. Abn-cbd binds GPR18 with an EC_{50} value of 835.8 nM (McHugh *et al.*, 2012), while it produces relaxation with EC_{50} value of 6.2 μM (Ho and Hiley, 2003a). As the concentrations needed for the vasodilator effects are far beyond the values sufficient to activate GPR18, the conclusion that the vascular effects of GPR18 agonists are indeed GPR18-dependent is rather speculative. Altogether, the data available do not yet allow the eCB receptor, the stimulation of which is required for endothelium-dependent relaxation to cannabinoids, to be classified as GPR18.

Sensitivity of the relaxant responses to pertussis toxin

In addition to sensitivity of the relaxant responses to the 'selective' antagonists of eCB receptors, the concept of the involvement of novel eCB receptors in the responses is largely supported by inhibition of cannabinoid-induced vasodilatation following pretreatment with pertussis toxin. However, a number of studies did not confirm the pertussis toxin sensitivity of mesenteric vasodilatation to Abn-CBD (Ho and Hiley, 2003a; Milman *et al.*, 2006), ARA-S (Milman *et al.*, 2006) and a synthetic cannabinoid-like compound VSN16 (3-(5-dimethylcarbamoyl-pent-1-enyl)-N-(2-hydroxy-1-methyl-ethyl)benzamide), which produces endothelium-dependent vasodilatation sensitive to rimonabant, O-1918 and AM-251 (Hoi *et al.*, 2007), thus questioning the involvement of a GPCR. Interestingly, unlike in the mesentery, the aortic relaxant effects of ARA-S and Abn-CBD in the study of Milman *et al.* were antagonized by pertussis toxin pretreatment.

However, the vasorelaxation was insensitive to O-1918 and rimonabant, commonly used selective antagonists of eCB receptors, suggesting distinct mechanisms for the vasodilatation in rat aorta and mesenteric artery (Milman *et al.*, 2006). In rat small mesenteric artery, pertussis toxin was shown to antagonize the relaxation to the Ca^{2+} ionophore A23187 (White and Hiley, 1997). The recently demonstrated multiplicity of GPR18- and GPR55-activated cellular pathways, which include either $G_{i/o}$- and $G\alpha_q$- (Console-Bram *et al.*, 2014) or $G\alpha_{12/13}$- and $G\alpha_q$- (Lauckner *et al.*, 2008) mediated signalling, respectively, may also partially explain the discrepancies in the reported sensitivity of eCB receptor-attributed relaxant responses to pertussis toxin.

Notably, the use of pertussis toxin in myographic studies as an indicator for GPCR involvement is associated with several flaws. Firstly, pertussis toxin is not a universal GPCR inhibitor but is only an inhibitor of the majority of the members of $G_{i/o}$ protein family. The ADP-ribosylating toxin, pertussis toxin, catalyzes the ADP-ribosylation of the α subunits of the heterotrimeric $G_{i/o}$ protein family ($G\alpha_i$, $G\alpha_o$ and $G\alpha_t$, but not $G\alpha_z$), thereby preventing the G proteins from interacting with the related GPCRs (Mangmool and Kurose, 2011). It should be noted that cannabinoid signalling is not limited to $G_{i/o}$ proteins and involves $G\alpha_s$ and $G\alpha_q$ proteins. Both CB_1 and CB_2 receptors activate multiple signal transduction pathways such as both inhibition and stimulation of adenylyl cyclase and protein kinase pathways via $G_{i/o}$ and $G\alpha_s$ proteins respectively (Bosier *et al.*, 2010). Coupling of the CB receptor to $G\alpha_q$ proteins results in stimulation of the activity of PLC (Bosier *et al.*, 2010), which hydrolyzes phosphatidylinositol 4,5-bisphosphate to diacyl glycerol and inositol 1,4,5-trisphosphate (IP_3) with subsequent activation of IP_3-operated Ca^{2+} permeable channels. Hence, pertussis toxin as a tool for detection of the involvement of $G\alpha_s$ and $G\alpha_q$ in the response has limited benefits, unless suppression of the signalling via $G_{i/o}$ proteins unmasks coupling of CB receptors to non-$G_{i/o}$ proteins. Secondly, pertussis toxin is not specific for $G_{i/o.}$ proteins and exhibits a number of GPCR-independent effects on key signalling mechanisms, including modulation of Ca^{2+} entry, cAMP and diacyl glycerol synthesis (Garcia *et al.*, 2001; Mangmool and Kurose, 2011), activation MAPK (Garcia *et al.*, 2001) and up-regulation of angiotensin type 1 (AT_1) receptor signalling (Nishida *et al.*, 2010). Consistent with these non-specific effects, pre-incubation of mesenteric arteries with pertussis toxin was found to inhibit endothelium-dependent relaxation to the Ca^{2+} ionophore A23187 (White and Hiley, 1997). Collectively, the sensitivity of the relaxant responses to pertussis toxin is likely to serve as an unreliable indicator for GPCR involvement.

In view of the conflicting pharmacology on the presence of eCB receptors, the data either supporting or non-supporting the concept of an eCB receptor was summarized in Tables 3 and 4 respectively.

Pertinent unsolved questions

Despite great efforts, our understanding of the vascular pharmacology of cannabinoid signalling and the mechanisms through which endocannabinoids affect vascular function is limited by assessment of vascular contractility as the technique of choice and utilization of non-vascular cell culture models. The variable effects of putative eCB receptor inhibitors and pertussis toxin indicate the complexity of the mechanisms of vascular cannabinoid signalling and possible simultaneous activation of several molecular targets, which may be expressed both in endothelial and smooth muscle cells. The largely accepted view on the presence of a novel CB receptor in vascular endothelium depends on key assumptions on the selectivity of eCB receptor agonists/antagonists, which appear to be rather premature. Recently identified multiple GPCR-independent targets for compounds, which have been used as selective agonists and antagonists for the eCB receptor, challenge the concept of the existence of an eCB receptor and its role in vascular responses to cannabinoids needs further support. Direct evidence for the existence of an eCB receptor is still missing and the mechanisms responsible for eCB receptor-attributed relaxation still need to be elucidated. Meanwhile, several key questions remain unanswered.

1. Do the vasodilator effects of cannabinoids commonly attributed to eCB receptors require a novel GPCR or do the effects, at least partially, depend on the stimulation of GPCR-independent targets? Clear dissection of GPCR-dependent and -independent endothelial signalling to cannabinoids at the level of ion channels and the molecular identity of the respective targets are ultimately required.
2. If a novel GPCR is involved in the vasodilatation induced by cannabinoids, is it only one or several eCB receptors? What is the molecular identity of the GPCRs and the respective signal transduction pathways? What is their role in vascular reactivity, angiogenesis and inflammation?
3. What are the multiple intracellular targets for endocannabinoids, the identity of these targets and their functional role?
4. Considering that in blood vessels, endothelial cell signalling and function is largely influenced by the smooth muscle cells, what are the molecular targets located on smooth muscle cells and their role in endothelial cell responses to cannabinoids?

Concluding remarks and future directions

In conclusion, substantial data have been accumulated suggesting that a site distinct from CB_1 and CB_2 receptors mediates the vasodilator effect of cannabinoids. There is evidence that cannabinoids bind to non-CB_1/CB_2 GPCRs, including GPR18 and GPR55, and their expression has been shown in vascular cells. Ligands and blockers of the putative eCB receptor widely used as selective compounds, often have GPCR-independent effects, influence functional properties of a number of ion channels/transporters expressed in the vasculature with potencies similar to that required to influence vascular reactivity, but higher than that required to activate respective GPCR. The observations that demonstrate Abn-CBD and NAGly are capable of activating GPR18 and inducing vasodilatation do not unequivocally indicate that the

Table 3

Data supporting the presence of a novel endothelial cannabinoid receptor

Read-out assay	Species and vascular bed	Agonist	pEC_{50}	Reference
PTX-sensitive CB_1/CB_2 receptor-independent endothelium-dependent relaxation	RSMA	AEA	6.3 μM	White and Hiley, 1997
	RSMA	NAGly	5.8 μM	Parmarand Ho, 2010
	RSMA	ARA-S	4.9 μM	Parmar and Ho, 2010
	RA	AEA	5.9 μM	Herradon *et al.*, 2007
	RA	THC	5.81 μM	O'Sullivan *et al.*, 2005b
	RA	Abn-CBD	not presented	Milman *et al.*, 2006
	RA	ARA-S	1–50 μM	Milman *et al.*, 2006
	RMA	Abn-CBD	5.6 μM	Offertaler *et al.*, 2003
	RabA	AEA	0.6 μM	Mukhopadhyay *et al.*, 2002
PTX-sensitive activation of p42/44 MAPK	Cell type	Agonist	Concentrations	Reference
	HUVEC	Abn-CBD	30 μM	Mo *et al.*, 2004
	HUVEC	Abn-CBD	10 μM	Offertaler *et al.*, 2003;
	HEK293-GPR18, BV-2	NAGly	10 nM–10 μM	McHugh *et al.*, 2010
	HEC-1B	NAGly, AEA	10 nM	McHugh *et al.*, 2012
PTX-sensitive cell migration	Cell type	Agonist	Concentrations	Reference
	HUVEC	Abn-CBD	10–50 μM	Mo *et al.*, 2004
	BV-2	NAGly	0.17 nM	McHugh *et al.*, 2010
	BV-2	Abn-CBD	13.1 nM	McHugh *et al.*, 2010
	HEK293-GPR18	NAGly	1 μM	McHugh *et al.*, 2010
	HEK293-GPR18	Abn-CBD	1 μM	McHugh *et al.*, 2010
Cell migration sensitive to PI3K/Akt inhibitors	Cell type	Agonist	Concentrations	Reference
	HUVEC	Abn-CBD	30 μM	Mo *et al.*, 2004

Abn-CBD, abnormal cannabidiol; AEA, anandamide; ARA-S, N-arachidonyl serine; BV-2, mouse microglial cells; CB_1R-cannabinoid receptor type 1; CB_2R-cannabinoid receptor type 2; PTX, pertussis toxin; RA, rat aorta; RabA, rabbit aorta; RMA, rat mesenteric artery; RSMA, rat small mesenteric artery; THC, Δ^9-tetrahydrocannabinol.

vasodilator effect of these compounds is mediated by GPR18. In this regard, it is unhelpful that due to a lack of selective GPR18 antagonists, O-1918 and cannabidiol are increasingly used to support the presence of eCB receptors in *in vitro* and *in vivo* experiments. Clearly, there is a need to attribute cannabinoid-induced vascular responses to stimulation of a specific orphan GPCR. To tackle this problem, it would be very helpful to develop and test selective agonists and antagonists of GPR18 and GPR55. Future studies on arteries isolated from GPR18 knockout mice would help to understand the role of GPR18 in the vasodilator effects of cannabinoids commonly attributed to eCB receptors. In addition, as endothelial electrical signalling has a key role in cell function and as there are limitations in cell culture models, systematic characterization of electrical events at the level of *in situ* vascular endothelium initiated by CB_1/CB_2 receptor-inactive cannabinoids with the identification of GPCR-dependent signalling is ultimately required. This would pinpoint the responses either to specific GPCR stimulation or GPCR-independent targets. It would be important to determine whether manipulations in GPR18 expression in excised vessels affect endothelial cell electrical responses and endothelium-dependent relaxation and other vascular functions. It is also important to extrapolate any signalling crosstalk between heterologously expressed GPR55 and CB_1/CB_2 receptors (Kargl *et al.*, 2012; Martinez-Pinilla *et al.*, 2014) and determine the biased agonism of GPR18 signalling reported for GPR18-expressing HEK293 cells (Console-Bram *et al.*, 2014) to native GPCRs expressed in the vascular endothelium.

In vascular preparations, molecular targets for cannabinoids may be located both on endothelial and smooth muscle cells. In the latter case, the signal initiated can be transmitted to the endothelial cell layer either through gap junctions or a diffusible messenger to produce an endothelium-dependent response. It is essential, therefore, to differentiate endothelium-derived signalling from that originating within the smooth muscle cells. Further work on understanding the mechanisms of the regulation of CB_1/CB_2 receptor-independent vascular effects of cannabinoids would provide further insights into the elucidation of the role of novel GPCRs sensitive to cannabinoids in health and disease.

Conflict of interest

None.

Table 4

Data non-supporting the presence of novel endothelial cannabinoid receptor

Read-out assay	Species/vascular bed	Agonist	pEC_{50}	Reference
PTX-insensitive relaxation to CB	RSMA	Abn-CBD	6.2 μM	Ho and Hiley, 2003a;
	RMA	Abn-CBD	2 μM	Milman *et al.*, 2006
	RMA	ARA-S	0.55 μM	Milman *et al.*, 2006
	RSMA	VSN16	88 nM	Hoi *et al.*, 2007
	RSMA	THC	5.37 μM	O'Sullivan *et al.*, 2005a
PTX-sensitive EDR to A23187	RSMA	A23187	6.46 μM	White and Hiley, 1997
			pEC_{50}	
Lack or minor EDR to CB	RabA	Abn-CBD	0.01–0.3 μM tested	Su and Vo, 2007
	RCA	AEA	6.8 μM	White *et al.*, 2001
	RSMA	AEA	6.3 μM	White and Hiley, 1997
	RMA	AEA	5.0 μM	O'Sullivan *et al.*, 2004
	RMA	AEA	0.3 μM tested	Randall *et al.*, 1996
	RA	AEA	5.9 μM	O'Sullivan *et al.*, 2005b
	RMA	AEA	6.2 μM	Harris *et al.*, 2002
	RSMA	AEA	6.7 μM	Ho and Hiley, 2003b
	RSMA	THC	5.37 μM	O'Sullivan *et al.*, 2005a
	RMA	THC	1–10 μM tested	O'Sullivan *et al.*, 2005a
			Rimonabant, μM	
Rimonabant inhibits vasodilatation to non-CB agonists/Ca^{2+} ionophores	RMA	Ionomycine	5 μM	Wagner *et al.*, 1999
	RCA	Bradykinin	1 μM	Randall *et al.*, 1997
	RMA	Bradykinin	1 μM	Randall *et al.*, 1996
	RMA	A23187	1 μM	Randall *et al.*, 1996
	RMA	Carbachol	1 μM	Randall *et al.*, 1996
	RSMA	Carbachol	1 μM	White and Hiley, 1997
			O-1918, μM	
No effect of O-1918 on EDR O-1918 inhibits endothelium-independent relaxation	RA	ARA-S	10 μM	Milman *et al.*, 2006
	MA	ARA-S	10 μM	Milman *et al.*, 2006
	RabPA	Abn-CBD	0.1–1 μM	Su and Vo, 2007
	RSMA	NAGly	3 μM	Parmar and Ho, 2010
	RSMA	SNP	3 μM	Parmar and Ho, 2010
GPCR-independent targets for O-1918	Targets	Effect	Concentration tested	
	NCX	Inhibition	10 μM	Bondarenko *et al.*, 2013
	BK_{Ca}	Inhibition	pIC_{50} = 6.6 μM	Godlewski *et al.*, 2009

Abn-CBD, abnormal cannabidiol; AEA, anandamide; ARA-S, N-arachidonyl serine; BK_{Ca}, high-conductance Ca^{2+}-dependent K^+ channels; CB, cannabinoid; EDR, endothelium-dependent relaxation; NCX, Na^+-Ca^{2+} exchanger; PTX, pertussis toxin; RA, rat aorta; RabA, rabbit aorta; RabPA, rabbit pulmonary artery; RCA, rat coronary artery; RMA, rat mesenteric artery; RSMA, rat small mesenteric artery; SNP, sodium nitroprussid; THC, Δ^9-tetrahydrocannabinol; VSN16, 3-(5-dimethylcarbamoyl-pent-1-enyl)-N-(2-hydroxy-1-methyl-ethyl)benzamide.

References

Alexander SP (2012). So what do we call GPR18 now? Br J Pharmacol 165: 2411–2413.

Alexander SP, Kendall DA (2007). The complications of promiscuity: endocannabinoid action and metabolism. Br J Pharmacol 152: 602–623.

Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013a). The Concise Guide to PHARMACOLOGY 2013/14: G protein-coupled receptors. Br J Pharmacol 170: 1459–1581.

Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013b). The Concise Guide to PHARMACOLOGY 2013/14: Ligand-gated ion channels. Br J Pharmacol 170: 1582–1606.

Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013c). The Concise Guide to PHARMACOLOGY 2013/14: Ion channels. Br J Pharmacol 170: 1607–1651.

Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013d). The Concise Guide to PHARMACOLOGY 2013/14: Nuclear hormone receptors. Br J Pharmacol 170: 1652–1675.

Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013f). The Concise Guide to PHARMACOLOGY 2013/14: Transporters. Br J Pharmacol 170: 1706–1796.

Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013g). The Concise Guide to PHARMACOLOGY 2013/14: Enzymes. Br J Pharmacol 170: 1797–1867.

Alsuleimani YM, Hiley CR (2013). Mechanisms of vasorelaxation induced by oleoylethanolamide in the rat small mesenteric artery. Eur J Pharmacol 702: 1–11.

Andrikopoulos P, Baba A, Matsuda T, Djamgoz MB, Yaqoob MM, Eccles SA (2011). Ca^{2+} influx through reverse mode Na^+/Ca^{2+} exchange is critical for vascular endothelial growth factor-mediated extracellular signal-regulated kinase (ERK) 1/2 activation and angiogenic functions of human endothelial cells. J Biol Chem 286: 37919–37931.

Baker D, Pryce G, Davies WL, Hiley CR (2006). In silico patent searching reveals a new cannabinoid receptor. Trends Pharmacol Sci 27: 1–4.

Barana A, Amorós I, Caballero R, Gómez R, Osuna L, Lillo MP *et al.* (2010). Endocannabinoids and cannabinoid analogues block cardiac hKv1.5 channels in a cannabinoid receptor-independent manner. Cardiovasc Res 85: 56–67.

Baranowska-Kuczko M, MacLean MR, Kozlowska H, Malinowska B (2012). Endothelium-dependent mechanisms of the vasodilatory effect of the endocannabinoid, anandamide, in the rat pulmonary artery. Pharmacol Res 66: 251–259.

Baur R, Gertsch J, Sigel E (2013). Do N-arachidonyl-glycine (NA-glycine) and 2-arachidonoyl glycerol (2-AG) share mode of action and the binding site on the β2 subunit of GABAA receptors? PeerJ 1: e149.

Bondarenko A (2004). Sodium-calcium exchanger contributes to membrane hyperpolarization of intact endothelial cells from rat aorta during acetylcholine stimulation. Br J Pharmacol 143: 9–18.

Bondarenko A, Sagach V (2006). Na^+-K^+-ATPase is involved in the sustained ACh-induced hyperpolarization of endothelial cells from rat aorta. Br J Pharmacol 149: 958–965.

Bondarenko A, Waldeck-Weiermair M, Naghdi S, Poteser M, Malli R, Graier WF (2010). GPR55-dependent and -independent ion signalling in response to lysophosphatidylinositol in endothelial cells. Br J Pharmacol 161: 308–320.

Bondarenko A, Panasiuk O, Stepanenko L, Goswami N, Sagach V (2012). Reduced hyperpolarization of endothelial cells following high dietary Na^+: effects of enalapril and tempol. Clin Exp Pharmacol Physiol 39: 608–613.

Bondarenko AI, Sagach VF (1997). Caffeine-induced electrical responses of intact endothelial cells. Neurophysiology 29: 156–161.

Bondarenko AI, Malli R, Graier WF (2011a). The GPR55 agonist lysophosphatidylinositol acts as an intracellular messenger and bidirectionally modulates Ca^{2+}-activated large-conductance K^+ channels in endothelial cells. Pflugers Arch 461: 177–189.

Bondarenko AI, Malli R, Graier WF (2011b). The GPR55 agonist lysophosphatidylinositol directly activates intermediate-conductance Ca^{2+}-activated K^+ channels. Pflugers Arch 462: 245–255.

Bondarenko AI, Drachuk K, Panasiuk O, Sagach V, Deak AT, Malli R *et al.* (2013). N-arachidonoyl glycine suppresses Na^+/Ca^{2+} exchanger-mediated Ca^{2+} entry into endothelial cells and activates BK channels independently of G-protein coupled receptors. Br J Pharmacol 169: 933–948.

Booz GW (2011). Cannabidiol as an emergent therapeutic strategy for lessening the impact of inflammation on oxidative stress. Free Radic Biol Med 51: 1054–1061.

Bosier B, Muccioli GG, Hermans E, Lambert DM (2010). Functionally selective cannabinoid receptor signalling: therapeutic implications and opportunities. Biochem Pharmacol 80: 1–12.

Brandes RP, Popp R, Ott G, Bredenkotter D, Wallner C, Busse R *et al.* (2002). The extracellular regulated kinases (ERK) 1/2 mediate cannabinoid-induced inhibition of gap junctional communication in endothelial cells. Br J Pharmacol 136: 709–716.

Brown AJ, Daniels DA, Kassim M, Brown S, Haslam CP, Terrell VR *et al.* (2011). Pharmacology of GPR55 in yeast and identification of GSK494581A as a mixed-activity glycine transporter subtype 1 inhibitor and GPR55 agonist. J Pharmacol Exp Ther 337: 236–246.

Bukoski RD, Batkai S, Jarai Z, Wang Y, Offertaler L, Jackson WF *et al.* (2002). CB_1 receptor antagonist SR141716A inhibits Ca^{2+}-induced relaxation in CB_1 receptor-deficient mice. Hypertension 39: 251–257.

Caldwell MD, Hu SS, Viswanathan S, Bradshaw H, Kelly ME, Straiker A (2013). A GPR18-based signaling system regulates IOP in murine eye. Br J Pharmacol 169: 834–843.

Chaytor AT, Martin PE, Evans WH, Randall MD, Griffith TM (1999). The endothelial component of cannabinoid-induced relaxation in rabbit mesenteric artery depends on gap junctional communication. J Physiol 520 (Pt 2): 539–550.

Console-Bram L, Brailoiu E, Brailoiu GC, Sharir H, Abood ME (2014). Activation of GPR18 by cannabinoid compounds: a tale of biased agonism. Br J Pharmacol 171: 3908–3917.

Deak AT, Groschner LN, Alam MR, Seles E, Bondarenko AI, Graier WF *et al.* (2013). The endocannabinoid N-arachidonoyl glycine (NAGly) inhibits store-operated Ca^{2+} entry by preventing STIM1-Orai1 interaction. J Cell Sci 126: 879–888.

Fleming I, Schermer B, Popp R, Busse R (1999). Inhibition of the production of endothelium-derived hyperpolarizing factor by cannabinoid receptor agonists. Br J Pharmacol 126: 949–960.

Fouad AA, Albuali WH, Al-Mulhim AS, Jresat I (2013). Cardioprotective effect of cannabidiol in rats exposed to doxorubicin toxicity. Environ Toxicol Pharmacol 36: 347–357.

Garcia JG, Wang P, Liu F, Hershenson MB, Borbiev T, Verin AD (2001). Pertussis toxin directly activates endothelial cell p42/p44 MAP kinases via a novel signaling pathway. Am J Physiol Cell Physiol 280: C1233–C1241.

Gasperi V, Dainese E, Oddi S, Sabatucci A, Maccarrone M (2012). GPR55 and its interaction with membrane lipids: comparison with other endocannabinoid-binding receptors. Curr Med Chem 20: 64–78.

Godlewski G, Offertaler L, Osei-Hyiaman D, Mo FM, Harvey-White J, Liu J *et al.* (2009). The endogenous brain constituent N-arachidonoyl L-serine is an activator of large conductance Ca^{2+}-activated K^+ channels. J Pharmacol Exp Ther 328: 351–361.

Harris D, McCulloch AI, Kendall DA, Randall MD (2002). Characterization of vasorelaxant responses to anandamide in the rat mesenteric arterial bed. J Physiol 539: 893–902.

Hassan S, Eldeeb K, Millns P, Bennett A, Alexander S, Kendall D (2014). Cannabidiol enhances microglial phagocytosis via transient receptor potential (TRP) channel activation. Br J Pharmacol 171: 2426–2439.

Henstridge CM, Balenga NA, Ford LA, Ross RA, Waldhoer M, Irving AJ (2009). The GPR55 ligand L-alpha-lysophosphatidylinositol promotes RhoA-dependent Ca^{2+} signaling and NFAT activation. FASEB J 23: 183–193.

Herradon E, Martin MI, Lopez-Miranda V (2007). Characterization of the vasorelaxant mechanisms of the endocannabinoid anandamide in rat aorta. Br J Pharmacol 152: 699–708.

Hiley CR, Hoi PM (2007). Oleamide: a fatty acid amide signaling molecule in the cardiovascular system? Cardiovasc Drug Rev 25: 46–60.

Hill AJ, Jones NA, Smith I, Hill CL, Williams CM, Stephens GJ *et al.* (2014). Voltage-gated sodium (Na) channel blockade by plant cannabinoids does not confer anticonvulsant effects per se. Neurosci Lett 566: 269–274.

Ho WS, Hiley CR (2003a). Vasodilator actions of abnormal-cannabidiol in rat isolated small mesenteric artery. Br J Pharmacol 138: 1320–1332.

Ho WS, Hiley CR (2003b). Endothelium-independent relaxation to cannabinoids in rat-isolated mesenteric artery and role of Ca^{2+} influx. Br J Pharmacol 139: 585–597.

Ho WS, Hiley CR (2004). Vasorelaxant activities of the putative endocannabinoid virodhamine in rat isolated small mesenteric artery. J Pharm Pharmacol 56: 869–875.

Ho WS, Yeung SYM (2009). Novel G-protein-coupled receptors in rat arteries: potential targets for N-arachidonoyl glycine? Proc Br Pharmacol Soc 7: 060P.

Hoi PM, Hiley CR (2006). Vasorelaxant effects of oleamide in rat small mesenteric artery indicate action at a novel cannabinoid receptor. Br J Pharmacol 147: 560–568.

Hoi PM, Visintin C, Okuyama M, Gardiner SM, Kaup SS, Bennett T *et al.* (2007). Vascular pharmacology of a novel cannabinoid-like compound, 3-(5-dimethylcarbamoyl-pent-1-enyl)-N-(2-hydroxy-1-methyl-ethyl)benzamide (VSN16) in the rat. Br J Pharmacol 152: 751–764.

Jarai Z, Wagner JA, Varga K, Lake KD, Compton DR, Martin BR *et al.* (1999). Cannabinoid-induced mesenteric vasodilation through an endothelial site distinct from CB_1 or CB_2 receptors. Proc Natl Acad Sci U S A 96: 14136–14141.

Jeong HJ, Vandenberg RJ, Vaughan CW (2010). N-arachidonyl-glycine modulates synaptic transmission in superficial dorsal horn. Br J Pharmacol 161: 925–935.

Johns DG, Behm DJ, Walker DJ, Ao Z, Shapland EM, Daniels DA *et al.* (2007). The novel endocannabinoid receptor GPR55 is activated by atypical cannabinoids but does not mediate their vasodilator effects. Br J Pharmacol 152: 825–831.

Kargl J, Balenga N, Parzmair GP, Brown AJ, Heinemann A, Waldhoer M (2012). The cannabinoid receptor CB1 modulates the signaling properties of the lysophosphatidylinositol receptor GPR55. J Biol Chem 287: 44234–44248.

Kim HI, Kim TH, Shin YK, Lee CS, Park M, Song JH (2005). Anandamide suppression of Na^+ currents in rat dorsal root ganglion neurons. Brain Res 1062: 39–47.

Kohler R, Brakemeier S, Kuhn M, Degenhardt C, Buhr H, Pries A *et al.* (2001). Expression of ryanodine receptor type 3 and TRP channels in endothelial cells: comparison of in situ and cultured human endothelial cells. Cardiovasc Res 51: 160–168.

Kohno M, Hasegawa H, Inoue A, Muraoka M, Miyazaki T, Oka K *et al.* (2006). Identification of N-arachidonylglycine as the endogenous ligand for orphan G-protein-coupled receptor GPR18. Biochem Biophys Res Commun 347: 827–832.

Kozlowska H, Baranowska M, Schlicker E, Kozlowski M, Laudanski J, Malinowska B (2007). Identification of the vasodilatory endothelial cannabinoid receptor in the human pulmonary artery. J Hypertens 25: 2240–2248.

Lauckner JE, Jensen JB, Chen HY, Lu HC, Hille B, Mackie K (2008). GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. Proc Natl Acad Sci U S A 105: 2699–2704.

Liu B, Song S, Jones PM, Persaud SJ (2014). GPR55: from orphan to metabolic regulator? Pharmacol Ther doi: 10.1016/j.pharmthera .2014.06.007. http://dx.doi.org/10.1016/j.pharmthera.2014.06.007

Liu J, Gao B, Mirshahi F, Sanyal AJ, Khanolkar AD, Makriyannis A *et al.* (2000). Functional CB1 cannabinoid receptors in human vascular endothelial cells. Biochem J 346 (Pt 3): 835–840.

Lopez-Miranda V, Dannert MT, Herradon E, Alsasua A, Martin MI (2010). Cytochrome P450 pathway contributes to methanandamide-induced vasorelaxation in rat aorta. Cardiovasc Drugs Ther 24: 379–389.

Lu VB, Puhl HL 3rd, Ikeda SR (2012). N-arachidonyl glycine (NAGly) does not activate G protein-coupled receptor 18 (GPR18) signaling via canonical pathways. Mol Pharmacol 83: 267–282.

MacIntyre J, Dong A, Straiker A, Zhu J, Howlett SE, Bagher A *et al.* (2014). Cannabinoid and lipid-mediated vasorelaxation in retinal microvasculature. Eur J Pharmacol 735C: 105–114.

Mangmool S, Kurose H (2011). G(i/o) protein-dependent and -independent actions of Pertussis Toxin (PTX). Toxins (Basel) 3: 884–899.

Marchenko SM, Sage SO (1993). Electrical properties of resting and acetylcholine-stimulated endothelium in intact rat aorta. J Physiol 462: 735–751.

Marchenko SM, Sage SO (1996). Calcium-activated potassium channels in the endothelium of intact rat aorta. J Physiol 492 (Pt 1): 53–60.

Martinez-Pinilla E, Reyes-Resina I, Onatibia-Astibia A, Zamarbide M, Ricobaraza A, Navarro G *et al.* (2014). CB and GPR55 receptors are co-expressed and form heteromers in rat and monkey striatum. Exp Neurol 261C: 44–52.

McCollum L, Howlett AC, Mukhopadhyay S (2007). Anandamide-mediated CB_1/CB_2 cannabinoid receptor–independent nitric oxide production in rabbit aortic endothelial cells. J Pharmacol Exp Ther 321: 930–937.

McHugh D, Hu SS, Rimmerman N, Juknat A, Vogel Z, Walker JM *et al.* (2010). N-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. BMC Neurosci 11: 44.

McHugh D, Page J, Dunn E, Bradshaw HB (2012). Delta(9)-Tetrahydrocannabinol and N-arachidonyl glycine are full agonists at GPR18 receptors and induce migration in human endometrial HEC-1B cells. Br J Pharmacol 165: 2414–2424.

Milman G, Maor Y, Abu-Lafi S, Horowitz M, Gallily R, Batkai S *et al.* (2006). N-arachidonoyl L-serine, an endocannabinoid-like brain constituent with vasodilatory properties. Proc Natl Acad Sci U S A 103: 2428–2433.

Mo FM, Offertaler L, Kunos G (2004). Atypical cannabinoid stimulates endothelial cell migration via a Gi/Go-coupled receptor distinct from CB_1, CB_2 or EDG. Eur J Pharmacol 489: 21–27.

Mukhopadhyay S, Chapnick BM, Howlett AC (2002). Anandamide-induced vasorelaxation in rabbit aortic rings has two

components: G protein dependent and independent. Am J Physiol Heart Circ Physiol 282: H2046–H2054.

Nicholson RA, Liao C, Zheng J, David LS, Coyne L, Errington AC *et al*. (2003). Sodium channel inhibition by anandamide and synthetic cannabimimetics in brain. Brain Res 978: 194–204.

Nishida M, Suda R, Nagamatsu Y, Tanabe S, Onohara N, Nakaya M *et al*. (2010). Pertussis toxin up-regulates angiotensin type 1 receptors through Toll-like receptor 4-mediated Rac activation. J Biol Chem 285: 15268–15277.

Offertaler L, Mo FM, Batkai S, Liu J, Begg M, Razdan RK *et al*. (2003). Selective ligands and cellular effectors of a G protein-coupled endothelial cannabinoid receptor. Mol Pharmacol 63: 699–705.

O'Sullivan SE, Kendall DA, Randall MD (2004). Heterogeneity in the mechanisms of vasorelaxation to anandamide in resistance and conduit rat mesenteric arteries. Br J Pharmacol 142: 435–442.

O'Sullivan SE, Kendall DA, Randall MD (2005a). The effects of Delta9-tetrahydrocannabinol in rat mesenteric vasculature, and its interactions with the endocannabinoid anandamide. Br J Pharmacol 145: 514–526.

O'Sullivan SE, Kendall DA, Randall MD (2005b). Vascular effects of delta 9-tetrahydrocannabinol (THC), anandamide and N-arachidonoyldopamine (NADA) in the rat isolated aorta. Eur J Pharmacol 507: 211–221.

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

Overton HA, Fyfe MC, Reynet C (2008). GPR119, a novel G protein-coupled receptor target for the treatment of type 2 diabetes and obesity. Br J Pharmacol 153 (Suppl. 1): S76–S81.

Oz M (2006). Receptor-independent actions of cannabinoids on cell membranes: focus on endocannabinoids. Pharmacol Ther 111: 114–144.

Parmar N, Ho WS (2010). N-arachidonoyl glycine, an endogenous lipid that acts as a vasorelaxant via nitric oxide and large conductance calcium-activated potassium channels. Br J Pharmacol 160: 594–603.

Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SP, Buneman OP *et al*.; NC-IUPHAR (2014). The IUPHAR/BPS Guide to PHARMACOLOGY: an expert-driven knowledgebase of drug targets and their ligands. Nucl. Acids Res 42 (Database Issue): D1098–106.

Pertwee RG (2010). Receptors and channels targeted by synthetic cannabinoid receptor agonists and antagonists. Curr Med Chem 17: 1360–1381.

Pertwee RG, Ross RA, Craib SJ, Thomas A (2002). (-)-Cannabidiol antagonizes cannabinoid receptor agonists and noradrenaline in the mouse vas deferens. Eur J Pharmacol 456: 99–106.

Randall MD, Kendall DA (1997). Involvement of a cannabinoid in endothelium-derived hyperpolarizing factor-mediated coronary vasorelaxation. Eur J Pharmacol 335: 205–209.

Randall MD, Alexander SP, Bennett T, Boyd EA, Fry JR, Gardiner SM *et al*. (1996). An endogenous cannabinoid as an endothelium-derived vasorelaxant. Biochem Biophys Res Commun 229: 114–120.

Randall MD, Harris D, Kendall DA, Ralevic V (2002). Cardiovascular effects of cannabinoids. Pharmacol Ther 95: 191–202.

Robson PJ (2013). Therapeutic potential of cannabinoid medicines. Drug Test Anal 6: 24–30.

Ross HR, Gilmore AJ, Connor M (2009). Inhibition of human recombinant T-type calcium channels by the endocannabinoid N-arachidonoyl dopamine. Br J Pharmacol 156: 740–750.

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

Sandow SL, Grayson TH (2009). Limits of isolation and culture: intact vascular endothelium and BK_{Ca}. Am J Physiol Heart Circ Physiol 297: H1–H7.

Shapira M, Vogel Z, Sarne Y (2000). Opioid and cannabinoid receptors share a common pool of GTP-binding proteins in cotransfected cells, but not in cells which endogenously coexpress the receptors. Cell Mol Neurobiol 20: 291–304.

Su JY, Vo AC (2007). 2-Arachidonylglyceryl ether and abnormal cannabidiol-induced vascular smooth muscle relaxation in rabbit pulmonary arteries via receptor-pertussis toxin sensitive G proteins-ERK1/2 signaling. Eur J Pharmacol 559: 189–195.

Takenouchi R, Inoue K, Kambe Y, Miyata A (2012). N-arachidonoyl glycine induces macrophage apoptosis via GPR18. Biochem Biophys Res Commun 418: 366–371.

Vang A, Mazer J, Casserly B, Choudhary G (2010). Activation of endothelial BKCa channels causes pulmonary vasodilation. Vascul Pharmacol 53: 122–129.

Wagner JA, Varga K, Jarai Z, Kunos G (1999). Mesenteric vasodilation mediated by endothelial anandamide receptors. Hypertension 33: 429–434.

Waldeck-Weiermair M, Zoratti C, Osibow K, Balenga N, Goessnitzer E, Waldhoer M *et al*. (2008). Integrin clustering enables anandamide-induced Ca^{2+} signaling in endothelial cells via GPR55 by protection against CB_1-receptor-triggered repression. J Cell Sci 121: 1704–1717.

White R, Hiley CR (1997). A comparison of EDHF-mediated and anandamide-induced relaxations in the rat isolated mesenteric artery. Br J Pharmacol 122: 1573–1584.

White R, Hiley CR (1998). The actions of the cannabinoid receptor antagonist, SR 141716A, in the rat isolated mesenteric artery. Br J Pharmacol 125: 689–696.

White R, Ho WS, Bottrill FE, Ford WR, Hiley CR (2001). Mechanisms of anandamide-induced vasorelaxation in rat isolated coronary arteries. Br J Pharmacol 134: 921–929.

Wilhelmsen K, Khakpour S, Tran A, Sheehan K, Schumacher M, Xu F *et al*. (2014). The endocannabinoid/endovanilloid N-arachidonoyl dopamine (NADA) and synthetic cannabinoid WIN55,212-2 abate the inflammatory activation of human endothelial cells. J Biol Chem 289: 13079–13100.

Wulff H, Kohler R (2013). Endothelial small-conductance and intermediate-conductance K_{Ca} channels: an update on their pharmacology and usefulness as cardiovascular targets. J Cardiovasc Pharmacol 61: 102–112.

Yang KH, Galadari S, Isaev D, Petroianu G, Shippenberg TS, Oz M (2010). The nonpsychoactive cannabinoid cannabidiol inhibits 5-hydroxytryptamine3A receptor-mediated currents in Xenopus laevis oocytes. J Pharmacol Exp Ther 333: 547–554.

Yang YY, Lin HC, Huang YT, Lee TY, Hou MC, Wang YW *et al*. (2007). Role of Ca2+-dependent potassium channels in in vitro anandamide-mediated mesenteric vasorelaxation in rats with biliary cirrhosis. Liver Int 27: 1045–1055.

Yin H, Chu A, Li W, Wang B, Shelton F, Otero F *et al*. (2009). Lipid G protein-coupled receptor ligand identification using beta-arrestin PathHunter assay. J Biol Chem 284: 12328–12338.

Zakrzeska A, Schlicker E, Baranowska M, Kozlowska H, Kwolek G, Malinowska B (2010). A cannabinoid receptor, sensitive to O-1918, is involved in the delayed hypotension induced by anandamide in anaesthetized rats. Br J Pharmacol 160: 574–584.

Zhang X, Maor Y, Wang JF, Kunos G, Groopman JE (2010). Endocannabinoid-like N-arachidonoyl serine is a novel pro-angiogenic mediator. Br J Pharmacol 160: 1583–1594.