British Journal of Pharmacology

British Journal of Pharmacology (2019) **176** 1395–1411 1395

Themed Section: 8th European Workshop on Cannabinoid Research

REVIEW ARTICLE

Cannabinoids in arterial, pulmonary and portal hypertension – mechanisms of action and potential therapeutic significance

Correspondence Professor Dr Barbara Malinowska, Department of Experimental Physiology and Pathophysiology, Medical University of Białystok, Mickiewicz Str. 2A, 15-222 Białystok, Poland. E-mail: bmalin@umb.edu.pl

Received 6 November 2017; **Revised** 24 January 2018; **Accepted** 5 February 2018

Barbara Malinowska[1], Marek Toczek[1], Anna Pędzińska-Betiuk[1] and Eberhard Schlicker[2]

[1]*Department of Experimental Physiology and Pathophysiology, Medical University of Białystok, Białystok, Poland, and* [2]*Department of Pharmacology and Toxicology, University of Bonn, Bonn, Germany*

The endocannabinoid system is overactivated in arterial, pulmonary and portal hypertension. In this paper, we present limited clinical data concerning the role of cannabinoids in human hypertension including polymorphism of endocannabinoid system components. We underline differences between the acute cannabinoid administration and their potential hypotensive effect after chronic application in experimental hypertension. We discuss pleiotropic effects of cannabinoids on the cardiovascular system mediated *via* numerous neuronal and non-neuronal mechanisms both in normotension and in hypertension. The final results are dependent on the model of hypertension, age, sex, the cannabinoid ligands used or the action *via* endocannabinoid metabolites. More experimental and clinical studies are needed to clarify the role of endocannabinoids in hypertension, not only in the search for new therapeutic strategies but also in the context of cardiovascular effects of cannabinoids and the steadily increasing legalization of cannabis use for recreational and medical purposes.

LINKED ARTICLES

This article is part of a themed section on 8th European Workshop on Cannabinoid Research. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v176.10/issuetoc

Abbreviations

2-AG, 2-arachidonoylglycerol; ACEA, arachidonyl-2-chloroethylamide; AEA, anandamide; AM1241, (2-iodo-5-nitrophenyl)-[1-[(1-methylpiperidin-2-yl)methyl]indol-3-yl]methanone; AM251, 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-piperidin-1-ylpyrazole-3-carboxamide; AM3506, 5-(4-hydroxyphenyl)pentane-1-sulfonyl fluoride; AM404, (5Z,8Z,11Z,14Z)-*N*-(4-hydroxyphenyl)icosa-5,8,11,14-tetraenamide; AM6545, 5-[4-(4-cyanobut-1-ynyl)phenyl]-1-(2,4-dichlorophenyl)-*N*-(1,1-dioxo-1,4-thiazinan-4-yl)-4-methylpyrazole-3-carboxamide; Ang II, angiotensin II; CB13, naphthalen-1-yl-(4-pentoxynaphthalen-1-yl)methanone; CBD, cannabidiol; CNR1, cannabinoid CB_1 receptor gene; CNR2, cannabinoid CB_2 receptor gene; CP55940, 2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-5-(2-methyloctan-2-yl)phenol; DOCA, 11-deoxycorticosterone acetate; eCBs, endocannabinoids; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; G3 vessels, third-order branches of the isolated mesenteric artery; HR, heart rate; HU210, (6aR,10aR)-9-(hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,10,10a-tetrahydrobenzo[*c*]chromen-1-ol; JWH-015, (2-methyl-1-propylindol-3-yl)-naphthalen-1-ylmethanone; JZL184, 4-nitrophenyl 4-[bis(2*H*-1,3-benzodioxol-5-yl)(hydroxy)methyl]piperidine-1-carboxylate; MethAEA, methanandamide; NTS, nucleus tractus solitarii; OMDM-2, (Z)-*N*-[(2R)-1-hydroxy-3-(4-hydroxyphenyl)propan-2-yl]octadec-9-enamide; PAH, pulmonary arterial hypertension; PEA, *N*-palmitoylethanolamine; PH, portal hypertension; PVN, paraventricular nucleus of hypothalamus; RAS, renin–angiotensin system; RSNA, renal sympathetic nerve activity; RVLM, rostral ventrolateral medulla; SHR, spontaneously hypertensive rats; SR144528, 5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-*N*-[(1S,4R,6S)-1,5,5-trimethyl-6-bicyclo[2.2.1]heptanyl]pyrazole-3-carboxamide; U46619, (Z)-7-[(1S,4R,5R,6S)-5-[(E,3S)-3-hydroxyoct-1-enyl]-3-oxabicyclo[2.2.1]heptan-6-yl]hept-5-enoic acid; URB597, 3-(3-carbamoylphenyl)phenyl *N*-cyclohexylcarbamate; WIN55212-2, (11R)-2-methyl-11-(morpholin-4-ylmethyl)-3-[(naphthalen-1-yl)carbonyl]-9-oxa-1-azatricyclo[6.3.1.0^{4,12}]dodeca-2,4,6,8(12)-tetraene; Δ^9-THC, Δ^9-tetrahydrocannabinol

DOI:10.1111/bph.14168

Introduction

Marijuana is the most widely used illicit drug, with approximately 200 million users worldwide (see Orsini *et al.*, 2016). **Δ⁹-Tetrahydrocannabinol** (Δ⁹-THC) is the major psychoactive component of the cannabis plant, but beyond the so-called phytocannabinoids, there are synthetic compounds, such as **nabilone** which is used for therapeutic purposes, and CB55940 and WIN55212-2 which are used for experimental purposes (for structures, see Malinowska *et al.*, 2012).

Another group of cannabinoids is the endogenously formed endocannabinoids (eCBs), including arachidonoyl ethanolamide [**anandamide** (AEA)] and **2-arachidonoylglycerol** (2-AG). They are formed from **arachidonic acid** and degraded by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase respectively. In addition, AEA is subject to an uptake process (Figure 1; see Cascio and Marini, 2015). The final component of this so-called eCB system (ECS) are receptors targeted by Δ⁹-THC, synthetic and eCBs, namely, the metabotropic cannabinoid CB_1 and CB_2 receptors. The eCBs have additional targets; the most well-known is the ionotropic **TRPV1** receptor, which is activated by AEA (Figure 1; see Pertwee, 2015). The ECS plays an important role both in a quantitative and qualitative manner. For example, the density of CB_1 receptors in various brain regions is higher than that of many other metabotropic receptors and these receptors are tonically active on numerous sites (see Pertwee, 2015).

At the moment, we are witnessing an increased legalization of cannabis use for recreational and medical purposes, particularly in the USA (Alshaarawy and Elbaz, 2016). For this reason, a clear-cut analysis of the risks of cannabis beyond the well-known addictive potential is important. For example, the ECS is implicated in cardiovascular functions, and there is concern that cannabis smoking increases cardiovascular disease risk in middle-aged adults (Hall, 2015). On the other hand, in their study dedicated to the role of the ECS in several hypertension models of the rat, Bátkai *et al.* (2004) reached the conclusion that 'targeting the ECS offers novel therapeutic strategies in the treatment of hypertension'.

The aim of the current study is to critically review the association of the ECS and increased BP levels and to consider the effects of cannabinoids on BP in hypertensive humans and animals.

Cannabinoids in human arterial hypertension

In humans, tachycardia is the most well known and consistent acute physiological change produced by cannabis preparations and also serves as a useful cannabinoid biomarker. This holds true for the major psychotropic cannabis constituent, Δ⁹-THC (whether delivered p.o., i.v. or through smoking), for the oromucosal spray Sativex® (containing Δ⁹-THC: cannabidiol (CBD) ≈ 1: 1) and for the orally administered synthetic cannabinoid nabilone (Cesamet®). Tachycardia may be accompanied by a modest increase in BP (particularly when supine) (see Malinowska *et al.*, 2012; Ho and Kelly, 2017). The combination of tachycardia and a decrease in BP in response to inhaled Δ⁹-THC has been described as well (Crawford and Merritt, 1979). The epidemiological estimates from the US National Health and Nutrition Examination Survey (NHANES; 2005–2012; n = 12 426)

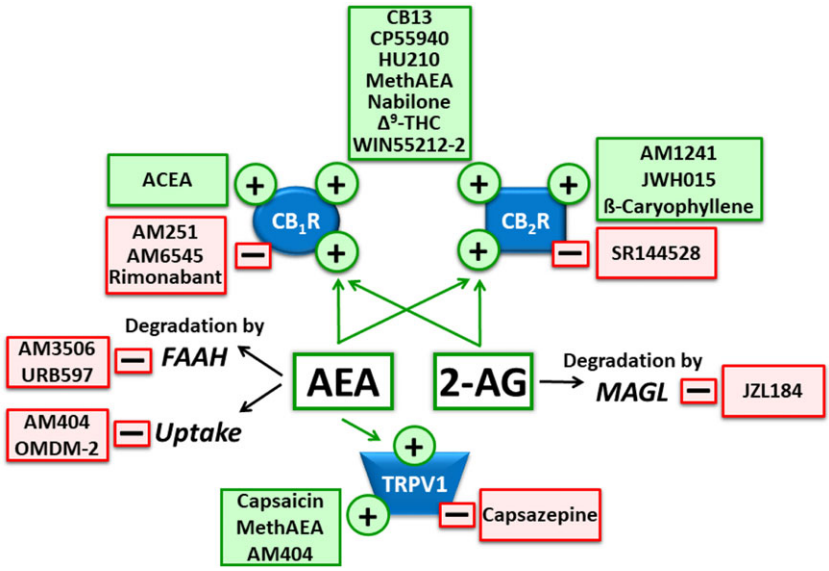

Figure 1

The ECS and modifying drugs. The ECS comprises (i) the eCBs themselves (AEA and 2-AG), (ii) enzymes for ligand biosynthesis and inactivation (in italics) and (iii) the receptor targets (blue structures). Only those components of the system and drugs that have been considered in this review, are shown in the Figure. Arrows (black line) designate chemical alteration or cellular uptake; arrows (green line) designate (partial) agonism at the respective receptor. The plus sign (and green colour) designates (partial) agonism at the respective receptor; the minus sign (and red colour) designates antagonism, inverse agonism or inhibition at the respective mechanism.

showed that, compared with "never use", "recent" cannabis use (at least once in the 30 days prior to the interview) was associated with higher systolic but not diastolic BP in an age-adjusted and sex-adjusted model (Alshaarawy and Elbaz, 2016). When administered alone, the non-psychoactive phytocannabinoid CBD decreased resting BP and the stress-induced increase in BP (Jadoon *et al.*, 2017). In contrast to acute administration, chronic use of cannabis may elicit a long-lasting bradycardia and prolonged reduction in BP (see Malinowska *et al.*, 2012). Abrupt cessation of heavy (Vandrey *et al.*, 2011) but not recreational (Bonnet, 2016), long-term daily cannabis use was followed by increases in BP.

Unfortunately, the possible involvement of eCBs in human arterial hypertension has been rarely investigated. No association was detected between cannabis use and hypertension or pre-hypertension in the NHANES studies (Alshaarawy and Elbaz, 2016). However, in the study by Crawford and Merritt (1979), differences between normotensive and hypertensive subjects were found but these were in small groups of subjects ($n = 8$ per group). Thus, the maximal positive chronotropic acute response to 2.8% Δ^9-THC inhalation was higher in sitting normotensive than in hypertensive patients, but the basal heart rate (HR) did not differ. By contrast, the intensity and duration of the depressor response to Δ^9-THC was greater in hypertensive than normotensive subjects. The latter difference may be, however, related to the different level of basal BP rather than to a specific alteration of the ECS.

A positive correlation between plasma levels of eCBs and BP has been found in hypertensive patients with obstructive sleep apnoea (Engeli *et al.*, 2012) and in depressed women (Ho *et al.*, 2012). Thus, the venous levels of AEA and the sum of 1-arachidonoylglycerol, 2-AG and **oleoylethanolamide** (but not arachidonic acid) were higher in sleep apnoea patients ($n = 55$) than in obese individuals ($n = 21$) without disordered nocturnal breathing. Importantly, AEA level was a stronger determinant of BP than sleep apnoea severity, obesity, insulin resistance and inflammation (Engeli *et al.*, 2012). Moreover, serum contents of AEA and 2-AG strongly and positively correlated with BP in depressed ($n = 28$) but not healthy ($n = 27$) women, suggesting that serum eCBs might compensate for an elevated BP in depression. However, an additional factor that might affect the final conclusion was that the depressed subjects were obese (with BMI ≥ 30 kg·m^{-2}), compared with their matched controls (Ho *et al.*, 2012).

A potential compensatory role of AEA in lowering BP has also been suggested for peritoneal dialysis patients during volume expansion ($n = 105$; Bai *et al.*, 2012). Thus, plasma AEA level was higher in patients with controlled hypertension (and high volume), as compared with patients with uncontrolled hypertension (and normal volume).

Genetic studies have partly confirmed the potential involvement of eCBs in hypertension. Thus, in a study including 1452 adult Japanese, male, but not female, carriers of the CC genotype of base 4895 in the 3′ untranslated region of exon 4 of the cannabinoid CB_1 receptor gene (CNR1), are more likely to be obese and hypertensive than the TT or TC allele carriers (Mutombo *et al.*, 2012). By contrast, a large case–control study of 1968 individuals from Germany showed that 13 common single-nucleotide polymorphisms in the cannabinoid CB_2 receptor gene (CNR2) were not significantly implicated in the development of classic cardiovascular risk factors including hypertension (Reinhard *et al.*, 2008). Interestingly, two FAAH defective gene variants exert different influence on BP. In young Caucasian students ($n = 215$) but not older male obese hypertensive patients ($n = 185$), the mutation associated with the exchange of Pro in position 129 by Thr led to a lower BP (Sarzani *et al.*, 2008). On the other hand, homozygous TT and heterozygous CT in position rs6703669 of the FAAH gene were associated with a higher BP in European adult men than homozygous CC ($n = 420$; Burgueño *et al.*, 2009).

Four large-scale multicentre phase III clinical trials [Rimonabant in Obesity (RIO)-Europe, RIO-Lipids, RIO-Diabetes and RIO-North America] were carried out to test the effect of the CB_1 receptor antagonist rimonabant (20 mg·day^{-1}; for 1 year combined with a moderately hypocaloric diet) on body weight (total $n = 6625$; 37% of patients had hypertension). Rimonabant reduced body weight and also decreased BP in hypertensive but not in normotensive obese patients, in a manner dependent on the body weight loss, suggesting that its hypotensive effect is related to the weight loss (Ruilope *et al.*, 2008). When rimonabant administration in the RIO-Europe Study was extended to 2 years, there was still a tendency towards a hypotensive effect, which, however, was no longer statistically significant ($n = 1507$; 41% with hypertension) (Van Gaal *et al.*, 2008).

Effects of acute cannabinoid administration on cardiovascular parameters in normotensive and hypertensive rats

In anaesthetized rats, rapid i.v. injection of AEA or its stable analogue methanandamide (MethAEA) induces typical triphasic changes in cardiovascular parameters: (I) a rapid, pronounced bradycardia and a transient drop in BP, (II) a brief pressor response and (III) a marked and prolonged decrease in BP and a slight decrease in HR. Only phases II and III occur after injection of Δ^9-THC and only phase III in response to WIN55212-2, CP55940 and HU210. By contrast, in conscious rats, AEA, MethAEA, Δ^9-THC and WIN55212-2 elicit a pressor response associated with vasoconstriction in the renal and mesenteric and vasodilatation in the hindquarters vascular bed as well as minor bradycardia (see Malinowska *et al.*, 2012; O'Sullivan, 2015; Ho and Kelly, 2017).

Cannabinoids (AEA, MethAEA and HU210) and inhibitors of FAAH (**URB597** and AM3506) or AEA transport (AM404 and OMDM-2) markedly decreased BP in anaesthetized or conscious rats with primary [spontaneously hypertensive (SHR)] and in different models of secondary hypertension. The same dose of the cannabinoid ligand did not affect BP, induced a modest decrease in BP only or even increased BP in the respective normotensive controls (Table 1). These depressor effects were connected with bradycardia (AEA and AM3506 but not URB597), decreased cardiac contractility and total peripheral resistance and renal, mesenteric and hindquarters vasodilatation. Again, these

Table 1

Effects of acute i.v. (if not stated otherwise) administration of cannabinoid ligands on cardiovascular parameters in hypertensive rats and their normotensive controls

Model of hypertension	Cannabinoid ligand	Dose ($mg \cdot kg^{-1}$)	Anaesthesia	Effects		References
				Hypertension	Normotension	
SHR	AEA	10	Pentobarbital	Sustained ↓ BP, ↓ HR, ↓ LVSP, ↓ dP/dt and ↓ TPR	WKY: modest ↓ BP and ↓ LVSP	Bátkai *et al.* (2004)
	HU210	0.001	Isoflurane	Sustained ↓ BP	WKY: modest ↓ BP	Godlewski *et al.* (2010)
	URB597	10	Pentobarbital	↓ BP, ↓ LVSP, ↓ dP/dt and ↓ TPR	WKY: no effect	Bátkai *et al.* (2004)
	AM3506	1	Isoflurane	Sustained ↓ BP, ↓ HR, ↓ LVSP, ↓ dP/dt, ↓ TPR, ↓ CO and ↓ [NAdr]	WKY: modest ↓ BP	Godlewski *et al.* (2010)
	AM404	10	Pentobarbital	↓ BP	WKY: no effect	Bátkai *et al.* (2004)
	OMDM-2	5				
	MethAEA	5	Conscious	(I) transient ↓ BP, (II) brief ↑ BP and (III) prolonged ↓ BP (phase III much stronger than in WKY)	WKY: (I) transient ↓ BP, (II) brief ↑ BP and (III) prolonged ↓ BP	Li *et al.* (2003)
	Δ^9-THC	5 p.o.	Conscious	↓ BP	↓ BP	Nahas *et al.* (1973)
Induced by Ang II	AEA	10	Pentobarbital	↓ BP	Sprague–Dawley: no effect	Bátkai *et al.* (2004)
	URB597	10				
Both SHR and hypertension induced by chronic NOS inhibition and acute hypertension induced by Ang II/AVP	AEA MethAEA WIN55212-2	3 3 0.15 or 0.25	Conscious	(I) ↑ BP, renal and mesenteric vasoconstriction and (II) ↓ BP, renal and mesenteric vasodilatation	↑ BP, renal and mesenteric vasoconstriction	Wheal *et al.* (2007a,b) and Ho and Gardiner (2009)
Induced by high-salt intake	MethAEA	5 or 15	Conscious	(I) transient ↓ BP, (II) brief ↑ BP and (III) prolonged ↓ BP (phase III much stronger than in WKY)	Wistar on normal-salt intake: (I) transient ↓ BP, (II) brief ↑ BP and (III) prolonged ↓ BP	Wang *et al.* (2007)
Induced by chronic inhibition of NOS	Δ^9-THC	1 infusion[b]	Conscious	↑ BP and ↓ HR, renal and mesenteric vasoconstriction and smaller	↑ BP, renal and mesenteric vasoconstriction	O'Sullivan *et al.* (2007)

continues

Table 1

(Continued)

Model of hypertension	Cannabinoid ligand	Dose ($mg \cdot kg^{-1}$)	Anaesthesia	Effects		References
				Hypertension	Normotension	
				hindquarters vasodilatation	and hindquarters vasodilatation	
1K1C	Δ^9-THC	1.5 i.p.	Conscious	↓ BP and ↓ HR	Not examined	Varma and Goldbaum (1975)
Transgenic (mRen-2)27	WIN55212-2	0.25	Conscious	↑ BP and ↓ HR, renal and mesenteric vasoconstriction and hindquarters vasodilatation	Sprague–Dawley: ↑ BP and ↓ HR, renal and mesenteric vasoconstriction	Gardiner *et al.* (2001)
	Rimonabant	10 p.o.	Conscious	↓ BP and ↓ HR	Sprague–Dawley: no effect	Schaich *et al.* (2014)
Both SHR and hypertension induced by chronic NOS inhibition and acute hypertension induced by Ang II/AVP	URB597	3	Conscious	No effect	No effect	Ho and Gardiner (2009) and Wheal *et al.* (2007a,b)
	AM251	1 or 3 both infusion[a]				
SHR	Rimonabant	3	Pentobarbital	↑ BP, ↑ LVSP, ↑ dP/dt and ↑ CO	WKY: no effect	Bátkai *et al.* (2004)
	SR144528	3	Pentobarbital	No effect	WKY: no effect	
Induced by Ang II	AM251	3	Pentobarbital	↑ BP	Sprague–Dawley: no effect	Bátkai *et al.* (2004)
Salt-sensitive Dahl rats kept on 8% NaCl	Rimonabant	3	Pentobarbital	↑ BP	Salt-sensitive Dahl rats kept on low-salt diet: no effect	Bátkai *et al.* (2004)

↑, increase; ↓, decrease; 1K1C, one-kidney one-clip; AVP, arginine vasopressin; CO, cardiac output; dP/dt, slope of systolic pressure increment; LVSP, left ventricular systolic pressure; [NAdr], plasma noradrenaline concentration; TPR, total peripheral resistance.

[a]Infusion of 2 $mL \cdot h^{-1}$ over 30 min.

[b]Infusion in 0.5 mL of vehicle over 20 min.

alterations occurred in hypertensive but not normotensive animals (Table 1). In conscious rats with hypertension induced by chronic inhibition of NOS the fall in BP in response to AEA, MethAEA and WIN55212-2 were connected with a vasodilatation in vascular beds and preceded by a pressor response, accompanied by renal and mesenteric vasoconstriction. Δ^9-THC given to SHR and renal hypertensive rats decreased BP and/or HR (see Table 1).

There are two exceptions from this pattern of cardiovascular responses to cannabinoid ligands (Table 1). First, infusion of Δ^9-THC into conscious rats made hypertensive by chronic inhibition of NOS and their normotensive controls caused comparable increases in BP associated with renal and mesenteric vasoconstriction; bradycardia occurred in hypertensive but not normotensive animals, and hindquarters vasodilatation was less in hypertensive than in normotensive rats. Second, in conscious hypertensive transgenic (mRen-2)27 rats and their normotensive controls, WIN55212-2 induced pressor and bradycardic responses was accompanied by renal and mesenteric vasoconstriction and hindquarters vasodilatation (the latter was less in hypertensive animals).

Neither cannabinoid receptor antagonists nor FAAH inhibitors nor genetic deletion of the latter components of the ECS affect BP or other cardiovascular parameters under physiological conditions (see Malinowska *et al.*, 2012; O'Sullivan, 2015; Ho and Kelly, 2017). By contrast, the following observations underline the involvement of the ECS in the regulation of the cardiovascular system in hypertension (Table 1). Firstly, FAAH inhibitors or inhibitors of the AEA transport exerted cardiovascular effects in hypertensive animals. Secondly, in rats made hypertensive by **angiotensin II** (Ang II)-**vasopressin** infusion, the FAAH inhibitor URB597 significantly prolonged the delayed hypotension induced by AEA and increased vasodilatation in the mesenteric vascular bed (Ho and Gardiner, 2009). Thirdly, in SHR and in most models of secondary hypertension (but not in their normotensive controls), bolus injection (but not infusion) of the CB_1 receptor antagonists rimonabant or **AM251** (but not of the CB_2 receptor antagonist **SR144528**) increased BP, HR and cardiac performance, that is, they produced effects opposite in direction to those elicited by the respective agonists. In hypertensive transgenic (mRen-2)27 rats in which WIN55212-2 increased BP and HR, treatment with rimonabant led to a decrease of both parameters (Table 1).

Effects of chronic cannabinoid administration on cardiovascular parameters in normotensive and hypertensive rats

Regrettably, there are only few papers describing the effect of chronic cannabinoid administration in hypertension. Thus, in SHR tolerance to the hypotensive effect of Δ^9-THC given at dose of 5 mg·kg^{-1} for 5 days or from 5 to 25 mg·kg^{-1} for 10 days developed within 2 and 9 days respectively (Nahas *et al.*, 1973). Interestingly, daily s.c. injection of Δ^9-THC 1 or 2 mg·kg^{-1} for 3 to 5 weeks prevented the development of renal hypertension (Varma and Goldbaum, 1975).

We have shown that administration of URB597 (1 mg·kg^{-1} twice daily) for 2 weeks to SHR and 11-deoxycorticosterone acetate (DOCA)-salt hypertensive rats (but not to their respective normotensive controls) caused an age-dependent and model-dependent effect. Thus, this regimen did not modify HR in any group but decreased BP (determined 12 h after the final dose of URB597) only in 11–12 but not 8- to 9-week-old DOCA-salt rats or in SHR. Moreover, URB597 reduced cardiac and renal hypertrophy in younger and hypertrophy of aorta and coronary vessels (but not mesenteric artery) in older DOCA-salt rats. However, it did not diminish organ hypertrophy in SHR as well as cardiomyocyte dimension and density of coronary blood vessels in DOCA-salt animals (Toczek *et al.*, 2016 and unpublished; Polak *et al.*, 2017b).

The eCB-related lipid, **N-palmitoylethanolamine (PEA)** 30 mg·kg^{-1}·day^{-1} given s.c. for 5 weeks to SHR strongly reduced BP during the last week (Mattace Raso *et al.*, 2015). Rimonabant 10 mg·kg^{-1}·day^{-1} given p.o. to transgenic (mRen-2)27 rats for 3 weeks showed a hypotensive effect from the beginning of its administration (Schaich *et al.*, 2014).

Potential mechanisms involved in the cardiovascular effects of endocannabinoids in arterial hypertension

Under physiological conditions, cannabinoids act on the cardiovascular system *via* numerous central and peripheral mechanisms (see Malinowska *et al.*, 2012; O'Sullivan, 2015; Ho and Kelly, 2017). Are the same mechanisms involved in the hypotensive action of cannabinoids in hypertension?

Role of CB_1 and TRPV1 receptors

The AEA-induced prolonged hypotension in normotensive rats is mediated *via* CB_1 receptors (Malinowska *et al.*, 2012; O'Sullivan, 2015; Ho and Kelly, 2017). Are these receptors also responsible for the hypotensive actions of cannabinoid ligands in hypertension (Figure 2)? Indeed, as already mentioned above for different hypertension models, the CB_1 receptor antagonists rimonabant or AM251 showed cardiovascular effects opposite in direction to those induced by agonists. Moreover, rimonabant and/or AM251 prevented or attenuated (i) the cardiovascular effects of the FAAH inhibitor AM3506 in anaesthetized SHR (Godlewski *et al.*, 2010), (ii) the haemodynamic responses to WIN55212-2 in conscious rats with acute hypertension induced by infusion of Ang II/vasopressin (Ho and Gardiner, 2009) and (iii) the pressor and vasoconstrictor effects of Δ^9-THC in conscious rats treated chronically with the NOS inhibitor **L-NAME** (O'Sullivan *et al.*, 2007b). The situation is more complicated for conscious SHR (Wheal *et al.*, 2007a). AM251 attenuated the pressor and vasoconstrictor effects of WIN55212-2 and the AEA-induced bradycardia, whereas the WIN55212-2-induced and AEA-induced hypotension and vasodilatation were not modified.

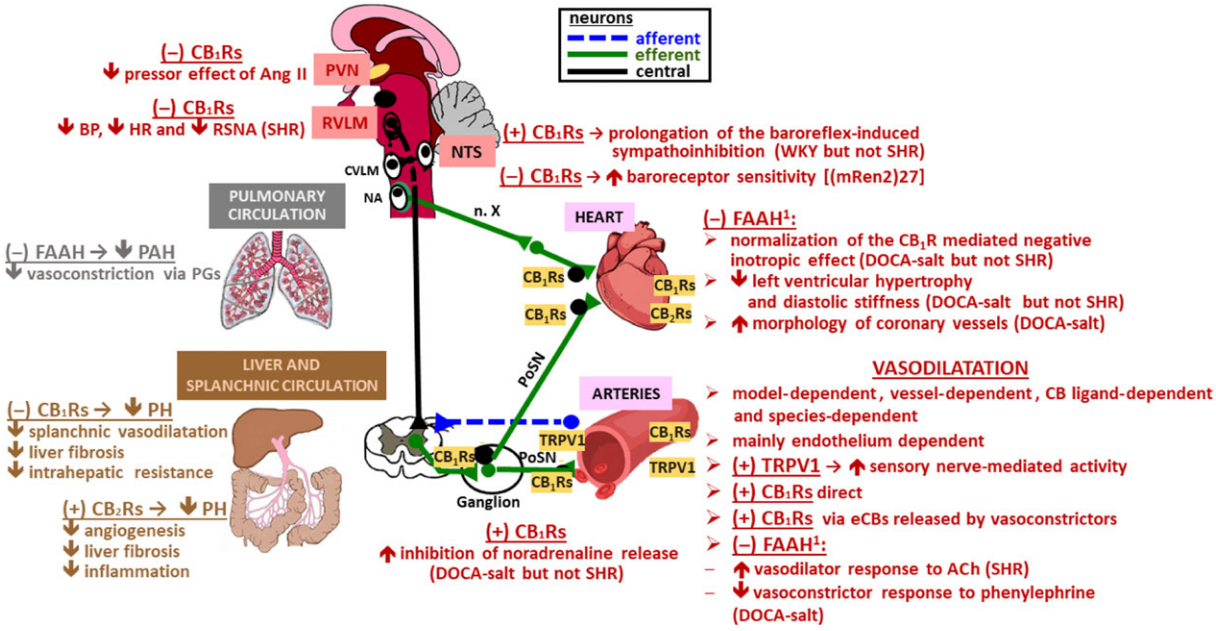

Figure 2

Involvement of the ECS in experimental arterial (red colours), pulmonary (grey colours) and portal (brown colours) hypertension. Activation (+) or blockade (–) of CB_1, CB_2 or TRPV1 receptors or of eCB degradation by FAAH have beneficial effects, dependent on the site and the type of hypertension. ↑, increase; ↓, decrease; CB, cannabinoid; CVLM, caudal ventrolateral medulla; DOCA, 11-deoxycorticosterone acetate; FAAH[1], 2 weeks of FAAH inhibition by URB597 1 mg·kg^{-1} twice daily; n. X, vagal nerve; NA, nucleus ambiguus; PoSN, postganglionic sympathetic nerve.

The eCB AEA does not only act on cannabinoid but also on vanilloid TRPV1 receptors (see Li *et al.*, 2003). TRPV1 receptors (which are also activated by the AEA uptake inhibitor AM404; Zygmunt *et al.*, 2000) occur on sensory nerves and mediate the vasodilator action of AEA *via* the release of **CGRP** (for literature, see Li *et al.*, 2003). Is this mechanism important also in hypertension (Figure 2)? The answer is yes, as the prolonged hypotensive effect of MethAEA in SHR (Li *et al.*, 2003) and in hypertensive rats fed a high-salt diet (Wang *et al.*, 2007) was blocked not only by rimonabant but also by the TRPV1 receptor antagonist **capsazepine**. Importantly, MethAEA increased plasma **CGRP** level in mesenteric arteries and capsazepine increased BP by itself, and both effects were stronger in rats on high-salt than those on normal diet. Moreover, dietary consumption of the TRPV1 receptor agonist capsaicin, over 1 year, decreased BP and improved endothelial function in SHR (Yang *et al.*, 2010). The pivotal role of vasodilatory TRPV1 receptors located on vascular sensory nerves in hypertension has also been confirmed in experiments on isolated vessels (see below). A role of TRPV1 receptors in hypertension is also suggested by the study of Marshall *et al.* (2013) on mice, although the protective effect of TRPV1 receptor deletion against obesity-induced hypertension and vascular hypertrophy is the opposite of what was expected.

Neuronal mechanisms in the central and peripheral nervous system

The involvement of central mechanisms in the cardiovascular effects of AM3506 has been clearly demonstrated in SHR (Godlewski *et al.*, 2010). Thus, the pronounced hypotension and bradycardia induced by this FAAH inhibitor were reduced by the CNS-penetrating CB_1 receptor antagonist AM251 but not by the peripherally restricted CB_1 receptor antagonist AM6545. Moreover, HU210 led to a much greater hypotension in SHR than in Wistar Kyoto (WKY) rats, whereas the non-brain penetrant CB_1/CB_2 receptor mixed agonist CB13 evoked similar hypotensive responses in both strains. Interestingly, CB_1 receptor density was similar in WKY rat and SHR brain but CB_1 receptor coupling efficiency was increased in brain membrane preparations from SHR compared with the WKY rats, suggesting that this effect might be responsible for the strong hypotensive effects of AM3506 and HU210 in SHR. These decreases in BP resulted probably from a CB_1 receptor-mediated decrease in sympathetic tone since they were (i) accompanied by a reduction in plasma noradrenaline levels and (ii) strongly reduced by ganglionic blockade with hexamethonium.

Sympathetic tone is mediated centrally by the rostral ventrolateral medulla (RVLM) and the paraventricular nucleus of the hypothalamus (PVN) (see Grzęda *et al.*, 2017; Wang *et al.*, 2017), two CNS nuclei, the function of which in hypertension is modified by cannabinoid ligands (Figure 2). Thus, it has been shown that microinjection of the CB_1 receptor agonist **arachidonyl-2-chloroethylamide** (ACEA) into the RVLM increased BP, HR and renal sympathetic nerve activity (RSNA). All the above changes were more pronounced and lasted longer in SHR than in normotensive controls. AM251 microinjected into the RVLM not only blocked all cardiovascular effects of ACEA but also decreased BP, HR and RSNA more markedly in SHR than in normotensive controls. Expression of CB_1 receptors was higher in the

RVLM of SHR, but levels of AEA and 2-AG were similar when compared with control rats (Wang *et al.*, 2017). In our own studies (Grzęda *et al.*, 2017 and unpublished) on anaesthetized rats, a CB_1 receptor-mediated depressor response could be evoked by microinjection of CP55940 into the PVN, which was reversed into a pressor response by i.v. AM251. The pressor response was enhanced in DOCA-salt hypertension but not in SHR, whereas the depressor response was not affected in either hypertensive model.

Sympathetic tone might also be diminished by the activation of presynaptic inhibitory CB_1 receptors, which are located on sympathetic nerve endings innervating resistance vessels and lead to a reduction of noradrenaline release (Figure 2; see Malinowska *et al.*, 2012). We have shown that the CB_1 receptor-dependent inhibitory effect of CP55940 on the neurogenic vasopressor response was enhanced in DOCA-salt hypertension but not in SHR (Toczek *et al.*, 2015; Malinowska *et al.*, 2017).

Baroreflex sensitivity is known to be diminished in hypertension (see Brozoski *et al.*, 2009). Microinjection of AEA into the nucleus tractus solitarii (NTS; the primary site for termination of baroreceptor afferent neurons) prolonged the baroreflex-related inhibition of RSNA in a manner dependent on CB_1 receptors (Figure 2). The expression of CB_1 receptors was reduced in SHR, in comparison with WKY rats. Accordingly, the baroreflex response evoked by AEA and the AEA uptake inhibitor AM404 was prolonged in normotensive, but not in hypertensive, rats (Brozoski *et al.*, 2009).

Rimonabant increased BP and cardiac contractility in SHR only after i.v. but not i.c.v. administration suggesting that its effects are related to a peripheral but not a central site of action (Bátkai *et al.*, 2004).

Non-neuronal peripheral mechanisms

Cannabinoids are known for their vasodilatory effects (see Stanley and O'Sullivan, 2014) that might play a protective role in hypertension. However, as shown in Table 2 (references therein refer to male rats if not stated otherwise), there are marked differences in terms of hypertension model, vascular bed and cannabinoid ligand. Thus, the vasodilatory response to AEA, MethAEA and CBD in third-order branches of the isolated mesenteric artery (G3 vessels) and/or in the perfused mesenteric bed was reduced in SHR but enhanced in hypertension induced by DOCA-salt or chronic inhibition of NOS. AEA-induced vasodilatation was enhanced in the isolated aorta of SHR. Moreover, the effect of Δ^9-THC but not AEA was enhanced in isolated G3 vessels in rats made hypertensive by NOS inhibition. Interestingly, in the latter model of hypertension, Δ^9-THC increased BP and decreased mesenteric vascular conductance (O'Sullivan *et al.*, 2007). The possible mechanisms responsible for the different responses to cannabinoid ligands in hypertension and the related references are listed in Table 2.

The potential hypotensive influence of cannabinoids might also result from the fact that they decrease the vasoconstriction or increase the vasodilatation induced by other agents (Table 2). Accordingly, CBD decreased the vasoconstrictor responses to **phenylephrine** and to the TXA_2 analogue **U46619** and increased the vasodilator effect of **ACh** and **sodium nitroprusside** in the aorta from SHR and DOCA-salt hypertensive rats when compared with their normotensive controls. Recently, a novel protective vascular mechanism of eCBs against various vasoconstrictors has been identified in the systemic and pulmonary circulations (Karpińska *et al.*, 2017). Thus, U46619, phenylephrine and Ang II may stimulate rapid endothelial release of eCBs, leading to a CB_1 receptor-dependent vasorelaxation, which *via* a negative feedback mechanism restrains vasoconstriction. We have detected this mechanism in G3 vessels but not in aorta isolated from SHR and WKY rats; the CB_1 receptor antagonist AM251 enhanced the vasoconstrictor response to phenylephrine and U46619 probably by preventing the vasodilatory effect of eCBs released by the latter vasoconstrictors (for details see Table 2).

Apart from factors such as the model of hypertension, vascular bed and cannabinoid ligand, sex-related effects have also been considered (Table 2). Thus, in SHR, the response to AEA in G3 vessels was decreased in hypertensive males, but not changed in female rats. Moreover, the vasodilator response to AEA was enhanced by **17β-oestradiol** in G3 vessels isolated from male but not female SHR or male WKY rat. On the other hand, **testosterone** did not modify the AEA effect in G3 vessels from female WKY rat and SHR. 17β-Oestradiol modifies FAAH, and exactly the same pattern of AEA response modification has been obtained in the presence of URB597, as seen with 17β-estradiol. Finally, the inhibitors of AEA and 2-AG degradation, URB597 and **JZL184**, respectively, diminished the vasoconstrictor response to Ang II in uterine artery isolated from rats in early stages of hypertensive pregnancy but not from normotensive controls (see Table 2).

We have examined whether the potential hypotensive effect of chronic FAAH inhibition by URB597 found in hypertensive DOCA-salt rats but not in SHR correlated with its vascular effects and/or changes in cardiac performance. The effect of chronic URB597 on BP only partly correlated with structural and functional changes in conductance and resistance vessels and was independent of **K_{Ca}2.3/K_{Ca}3.1** channel-based vasodilatation. Thus, URB597 did not modify the response to MetAEA in G3 vessels from both hypertensive models. However, it reduced the vasoconstrictor effect of phenylephrine in DOCA-salt, but not in SHR. On the other hand, it enhanced the vasodilatory effect of ACh in SHR but not in DOCA-salt rats and reduced hypertrophy of aorta (but not G3) in DOCA-salt but not in SHR (see Table 2).

Reduction of increased cardiac contractility in hypertension could be one of the major components of the antihypertensive action of acute injection of FAAH inhibitors (Bátkai *et al.*, 2004; Godlewski *et al.*, 2010). Thus, in DOCA-salt but not in SHR, chronic URB597 administration reduced left ventricular hypertrophy and diastolic stiffness enhanced in hypertension. Importantly, it normalized the negative inotropic effect of CP55940 in left atria and the positive inotropic influence of **isoprenaline** in isolated heart both of which were diminished in hypertension. By contrast, in SHR, only normalization of the positive inotropic effect of isoprenaline was observed (Pędzińska-Betiuk *et al.*, 2017). Moreover, chronic FAAH inhibition improved the morphology of coronary vessels and myocardial glucose metabolism and slightly modified lipid metabolism in DOCA-salt hypertension (Polak *et al.*, 2017a,b).

Table 2

Effects of hypertension on the vascular effects of cannabinoid ligands in isolated vessels or perfused vascular beds taken from male (if not stated otherwise) hypertensive rats

Model of hypertension	Cannabinoid ligand	Plus other CB or non-CB agents	Vascular preparation	Degree of vasodilatation (if not stated otherwise) in comparison with the respective control	Mechanisms		References
					Involved	Excluded	
SHR	AEA	–	Mesenteric arterial bed[b]	↓	↓ NO-dependent relaxation and ENDO	Changes in sensory nerve activity	Wheal and Randall (2009)
	MethAEA	–	G3[a]	↓	H: CB_1	–	Kloza *et al.* (2017a)
	AEA	–	G3[a]	Male: ↓ and female: ↔ (H: in female stronger than in male)	TRPV1 and ENDO	–	Ho (2013)
	AEA	–	Thoracic aorta[a]	↑	ENDO	CB_1 and TRPV1	Wheal and Randall (2009)
	CBD	–	G3[a]	↓	–	–	Baranowska-Kuczko *et al.* (2016b)
	Oleamide	–	Abdominal aorta[a]	↑	PG	NO, FAAH, TRPV1 and ENDO	Hopps *et al.* (2012)
Induced by chronic inhibition of NOS	AEA	–	G3[a]	↔	–	–	Wheal *et al.* (2007b)
	AEA	–	Mesenteric arterial bed[b]	↑	↑ sensory nervemediated activity	–	Wheal *et al.* (2007b) and Wheal and Randall (2009)
	AEA	–	Thoracic aorta[a]	↔	–	CB_1, TRPV1, NO and PG	Wheal and Randall (2009)
	Δ^9-THC	–	Aorta[a]	↔	–	–	O'Sullivan *et al.* (2007)
	Δ^9-THC	–	G0[a]	↑	–	–	O'Sullivan *et al.* (2007)
			G3[a]	↑	↑ sensory nerve-mediated activity TRPV1, H: PG	CB_1	
2K1C	AEA	–	Thoracic aorta[a]	↑	CB_1, CB_2, ↑ phosphorylation of endothelial NOS and ENDO	–	Guo *et al.* (2015)

continues

Table 2

(Continued)

Model of hypertension	Cannabinoid ligand	Plus other CB or non-CB agents	Vascular preparation	Degree of vasodilatation (if not stated otherwise) in comparison with the respective control	Mechanisms		References
					Involved	Excluded	
DOCA-salt	MethAEA	–	G3[a]	↑	TRPV1 and H: CB_1	–	Baranowska-Kuczko *et al.* (2016a)
	CBD	–	G3[a]	↑	–	–	Baranowska-Kuczko *et al.* (2016b)
SHR	AM251	Phe U46619	G3[a] but not in aorta[a]	↑ contraction induced by Phe and U46619	CB_1	–	Kloza *et al.* (2017a)
Both DOCA-salt and SHR	URB597 (chronic)	MethAEA	G3[a]	No changes in the vasodilator response to MethAEA	–	–	Baranowska-Kuczko *et al.* (2016a, 2017) and Kloza *et al.* (2017a)
	URB597 (chronic)	Phe	G3[a] but not in aorta[a]	↓ contraction induced by Phe in DOCA-salt (but not in SHR)	–	–	
	URB597 (chronic)	Ach	G3[a] Aorta [a]	H: ↑ vasodilator response to Ach in SHR (but not in DOCA-salt)	CB_1	–	
DOCA-salt	URB597 (chronic)	ACh	G3[a]	No changes in non-NO, non-PG-mediated relaxation in the presence of K_{Ca} channel blockers	–	–	Kloza *et al.* (2017b)
	URB597 (chronic)	NS309	G3[a]	No changes in the vasodilator response to NS309	–	–	
	CBD	Phe U46619	Aorta[a]	↓ contraction induced by Phe and U46619	ENDO	–	Baranowska-Kuczko *et al.* (2016a, 2017)
	CBD	ACh	Aorta[a]	↑ vasodilator response to ACh	ENDO	–	

continues

Table 2

(Continued)

Model of hypertension	Cannabinoid ligand	Plus other CB or non-CB agents	Vascular preparation	Degree of vasodilatation (if not stated otherwise) in comparison with the respective control	Mechanisms		References
					Involved	Excluded	
SHR	AEA	17β-Oestradiol	G3[a]	↑ vasodilator response to AEA in male but not in female, similarly in WKY	TRPV1	ENDO	Ho (2013)
	AEA	Testosterone	G3[a]	Female: no change in the vasodilator response to AEA	–	ENDO	
	AEA	URB597	G3[a]	↑ vasodilator response to AEA in male but not in female, similarly in WKY	ENDO	–	
Hypertensive pregnancy	URB597 JZL184	Ang II	Uterine artery	H: ↓ contraction induced by Ang II	CB_1 and ENDO	–	Pulgar *et al.* (2014)

↔ no changes; ↑ increase; ↓ decrease; CB, cannabinoid; ENDO, endothelium dependent; G0 and G3, superior mesenteric arteries and their third-order (small resistance) branches respectively; H, mechanisms determined in hypertension but not in normotension; 2K1C, two-kidney one-clip; NS309, activator of potassium $K_{Ca}2.3/K_{Ca}3.1$ channels; Phe, phenylephrine (their involvement determined by the use of the COX inhibitor indomethacin).

[a]Isolated vessels taken from male (if not stated otherwise) hypertensive rats.

[b]Perfused vascular beds taken from male (if not stated otherwise) hypertensive rats.

Interaction of the endocannabinoid with the renin–angiotensin system

There is an increasing number of reports linking the ECS to the renin–angiotensin system (RAS). Interestingly, the cardiovascular effects of rimonabant in RAS-dependent hypertensive (mRen2)27 rats were opposite in direction to those observed in SHR (Table 1). Thus, its acute and chronic oral administration reduced rather than increased BP and HR in (mRen2)27 rats (Schaich *et al*., 2014). Acutely administered rimonabant might decrease BP *via* CB_1 receptors located in the PVN, because its microinjection into this nucleus prevented the pressor effect of Ang II given into the PVN (determined in normotensive rats only; Gyombolai *et al*., 2012). The reason for the hypotensive effect after chronic CB_1 receptor blockade may be the enhancement of baroreceptor sensitivity including an increase and decrease in the parasympathetic and sympathetic components respectively (Schaich *et al*., 2014). By contrast, CB_1 receptor stimulation in the NTS of WKY rat but not SHR prolonged the baroreflex response (see above; Brozoski *et al*., 2009). Moreover, microinjection of rimonabant into the NTS did not modify baroreceptor sensitivity in normotensive rats, decreased it in ASrAOGEN rats with low glial angiotensinogen and increased it in (mRen2)27 rats with up-regulated brain RAS expression, that is, animals with enhanced and impaired resting baroreceptor sensitivity respectively. The level of 2-AG (but not AEA) was increased in the NTS of (mRen2)27 but not of other rat strains, suggesting that an up-regulated brain ECS in Ang II-dependent hypertension may contribute to the impaired baroreceptor sensitivity in this model of hypertension (Schaich *et al*., 2016).

An interesting interaction in brainstem astrocytes has been determined between Ang II and CB_1 receptors, which have been shown to elevate and to neutralize the inflammatory state respectively. Basal expression of CB_1 receptors in brainstem astrocytes, which may play a role in hypertension (Haspula and Clark, 2016), was lower for SHR than for Wistar rats. Ang II infusion further decreased CB_1 receptor expression *via* **AT_1 receptor**s. The authors have also examined cerebellar astrocytes, which may play a role in attention-deficit hyperactivity disorder. In this set of experiments, basal expression of CB_1 receptors was higher in SHR than in WKY rats. Ang II infusion further increased CB_1 receptor expression *via* both AT_1 and **AT_2 receptor**s.

Moreover, the AEA transporter activity that is dependent on AT_1 receptors and an increase in ROS production was decreased by Ang II in SHR when compared with WKY rats (Shi *et al*., 2012). The protective role of CB_1 receptors against the vasoconstrictor effect of Ang II in uterine artery isolated from rats with hypertensive pregnancy has been described above (Pulgar *et al*., 2014).

The endocannabinoid system in pulmonary and portal hypertension

Pulmonary arterial hypertension (PAH) is a progressive, potentially lethal disease. The eCBs are known as potent vasodilators of isolated human and rat pulmonary arteries (see Stanley and O'Sullivan, 2014). However, their potential significance in PAH therapy is still to be established. On the one hand, AEA, which has an inhibitory effect on the two-pore domain potassium (K2P) channel, has been shown to reduce the hypoxia-induced vasoconstriction (one of the pathogenetic factors in PAH) in murine intra-acinar and pre-acinar arteries and had virtually no effect on the vascular calibre under normoxia (Murtaza *et al*., 2017). On the other hand, in the isolated perfused lung of the mouse (Wenzel *et al*., 2013) and the rabbit (Wahn *et al*., 2005), AEA increased the perfusion pressure *via* COX-2-generated metabolites, following enzymic degradation by FAAH into arachidonic acid products (Figure 2). Accordingly, the onset of PAH was prevented in $FAAH^{-/-}$ mice or by treating wild-type mice with the FAAH inhibitor URB597 for 3 weeks of hypoxia (Wenzel *et al*., 2013). The reason why AEA acts so differently in isolated pulmonary vessels and in perfused lungs is unclear, and the potential role of eCBs in PAH should be further investigated.

Portal hypertension (PH) is one of the most severe complications of liver cirrhosis, which in turn is a leading cause of death and liver transplantation. In this haemodynamic disturbance, mesenteric splanchnic vasodilation and hepatic vascular resistance play an essential role (see Baldassarre *et al*., 2013; Yang and Lin, 2015). The ECS is up-regulated in human and experimental cirrhosis, as a higher plasma AEA level was found in patients (also increased PEA and **oleamide** levels; Caraceni *et al*., 2010) and in rats with experimental cirrhosis (see Baldassarre *et al*., 2013; Yang and Lin, 2015). AEA caused a stronger CB_1 and TRPV1 receptor-dependent vasodilatation of mesenteric, but not femoral, arteries isolated from cirrhotic rats, in comparison with their controls (Domenicali *et al*., 2005). On the other hand, AEA caused vasoconstriction in the isolated perfused liver, which was more marked for rats with cirrhosis induced by bile duct ligation than for their controls. The increased intrahepatic resistance was correlated with the release of TXA_2 (Yang and Lin, 2015). Blockade of CB_1 receptors by acute or chronic rimonabant administration and/or CB_1 receptor deficiency increased the splanchnic vascular resistance in cirrhotic animals, leading to a concomitant decrease in the mesenteric arterial blood flow, reduced liver fibrosis and finally decreased PH and/or improved survival in different models of chronic liver damage. Opposing effects were found in CB_2 receptor-deficient mice with experimental cirrhosis or in patients with the Q63R variant of the CB_2 receptor that impairs its immunomodulating function (see Baldassarre *et al*., 2013). Accordingly, the CB_2 receptor agonist **JWH015** reduced PH, superior mesenteric arterial blood flow, hepatic angiogenesis, portosystemic shunting and hepatic fibrosis in biliary cirrhotic rats. The vascular effects in this model were, at least in part, connected with COX and NOS down-regulation (Huang *et al*., 2012). In the study by Reichenbach *et al*. (2012), the CB_2 receptor agonist **AM1241** led to similar beneficial effects on rats with CCl_4-induced liver fibrosis (Figure 2).

A beneficial effect on cirrhotic disturbances might also be achieved by reduction of eCBs levels. Thus, the administration of the antibiotic ciprofloxacin resulted not only in a decrease in endotoxemia but also in a fall in the hepatic

Table 3

Changes of the endocannabinoid system in hypertension (in comparison with respective normotensive groups)

Model of hypertension	Age	eCB levels	Receptor expression (or coupling efficiency)	Enzyme expression or activity and AEA transporter activity[a]	References
Hypertensive patients	48–65 years	Plasma: ↑ AEA	–	↓ AEA transporter activity	Li *et al.* (2009)
SHR	16 weeks	Plasma: ↑ AEA	–	↓ AEA transporter activity	Li *et al.* (2009) and Shi *et al.* (2012)
	32–40 weeks	Heart: ↓ AEA and ↔ 2-AG	Heart: ↑ CB_1 Aortic endothelium: ↑ CB_1	Heart: ↑ FAAH expression	Bátkai *et al.* (2004)
	12–16 weeks	Brain: ↔ AEA and ↔ 2-AG	Brain: ↑ CB_1 (coupling efficiency)	Brain: ↔ FAAH activity	Godlewski *et al.* (2010)
	10–12 weeks	–	Heart (LV): ↔ CB_1 and ↔ CB_2	Heart (LV): ↓ FAAH expression	Pędzińska-Betiuk *et al.* (2017)
	10–12 weeks	–	Mesenteric artery: ↑ CB_1	–	Kloza *et al.* (2017a)
	10–12 weeks	Kidney: ↑ AEA and ↑ 2-AG	Kidney: ↑ CB_1, ↑ CB_2 and ↓ TRPV1	Kidney: ↑ FAAH activity and ↑ MAGL activity	Biernacki *et al.* (2017)
DOCA-salt	13–14 weeks	–	Aorta: ↔ CB_1 and ↔ TRPV1 Mesenteric artery: ↑ CB_1 and ↔ TRPV1	Aorta: ↔ FAAH expression Mesenteric artery: ↔ FAAH expression	Baranowska-Kuczko *et al.* (2016a,b)
	13–14 weeks	–	Heart (LV): ↓ CB_1 and ↓ CB_2	Heart (LV): ↓ FAAH expression	Pędzińska-Betiuk *et al.* (2017)
	11–12 weeks	Liver: ↑ AEA and ↑ 2-AG	Liver: ↑ CB_1, ↔ CB_2 and ↔ TRPV1	Liver: ↔ FAAH activity and ↑ MAGL activity	Biernacki *et al.* (2016)
	10–12 weeks	Kidney: ↑ AEA and ↑ 2-AG	Kidney: ↑ CB_1, ↑ CB_2 and ↓ TRPV1	Kidney: ↑ FAAH activity and ↑ MAGL activity	Biernacki *et al.* (2017)
High-salt intake	9 weeks	Plasma: ↑ AEA	Mesenteric artery: ↑ TRPV1	–	Wang *et al.* (2007)
2K1C	10–11 weeks	Plasma: ↑ AEA	–	↔ AEA transporter activity	Li *et al.* (2009)

↔ no change; ↑ increase; ↓ decrease; 2K1C, two-kidney one-clip; $CB_{1,2}$, cannabinoid CB_1, CB_2 receptor; LV, left ventricle.

[a]AEA transporter activity was determined in lymphocytes.

AEA and 2-AG levels accompanied by an improvement of hepatic microcirculation and PH, and a decrease and increase in the expression of CB_1 and CB_2 receptors respectively (see Baldassarre *et al.*, 2013).

Changes in the endocannabinoid system in hypertension

The following changes in components of ECS demonstrated that it plays a protective role in hypertension (see Table 3). Firstly, the plasma level of AEA was higher in hypertensive patients and in three models of hypertensive rats (SHR, high-salt diet and two-kidney one-clip) when compared with the respective normotensive controls. Secondly, the expression of CB_1 and TRPV1 receptors, activation of which leads to vasodilatation (CB_1 and TRPV1 receptors) and a decrease in cardiac contractility (CB_1 receptors), was higher in the heart of SHR, aorta of SHR and mesenteric artery of SHR, DOCA-salt rats and rats fed a high-salt diet. These changes were dependent on the hypertension model and the tissue. Thus, unlike in the plasma, the AEA level was decreased in hearts from SHR. In DOCA-salt hypertensive rats, expression of CB_1 receptors was increased in G3 vessels but not in the aorta. The higher level of CB_1 receptors in the total heart of SHR (Bátkai *et al.*, 2004) but not in their left ventricles (Pędzińska-Betiuk *et al.*, 2017) might result from the different age or from regional differences within the heart. One should keep in mind that cardiac CB_1 receptors are known to exert pro-inflammatory, pro-oxidative and pro-fibrogenic effects, whereas the CB_2 receptors diminish the above actions (see Ho and Kelly, 2017); TRPV1 receptors attenuated the high-salt-induced cardiac hypertrophy (Lang *et al.*, 2015).

The question arises as to which mechanism is responsible for the enhancement of eCB levels in hypertension. One possibility is the decrease in activity and/or expression of the enzymes responsible for their degradation. Accordingly, the decrease in myocardial AEA level in SHR was connected with an increase in FAAH expression in this tissue, whereas in the brain, neither AEA level nor FAAH expression was changed. The central hypotensive effect of the FAAH inhibitor AM3506 was associated with an increase in brain CB_1 receptor coupling. Another mechanism might be the attenuation of AEA transporter activity, which may explain the higher plasma AEA level in hypertensive patients and SHR, but not in renal hypertensive rats (for details see Table 3).

Conclusions

In conclusion, the ECS is overactivated in arterial, pulmonary and portal hypertension. It is not clear, however, whether these changes are protective or detrimental, as cannabinoids exert pleiotropic effects on the cardiovascular system *via* numerous central and peripheral mechanisms, in both normotension and in hypertension. In addition, vasodilatation of the mesenteric vascular bed is protective in arterial, and detrimental in portal hypertension. Thus, as shown in Figure 2, the activation of central and peripheral CB_1 receptors leads mainly to an increase and decrease in arterial BP respectively. Effects on BP might be connected with a central and peripheral modification of sympathetic tone, cardiac contractility and vascular diameter. One should keep in mind that eCBs can cause vasodilatation directly and vasoconstriction *via* formation of metabolites (PGs). This is one of the reasons for the different results obtained *in vitro* in isolated vessels and in perfused vascular beds (e.g. in lungs). The final results are additionally dependent on the model of hypertension, age, sex and the cannabinoid ligands used. Importantly, chronic experiments have cast doubt on the promising suggestion derived from acute experiments that targeting the ECS offers novel therapeutic strategies in the treatment of hypertension. Additional factors responsible for the modification of BP in chronic experiments include the increase and decrease of inflammatory, oxidative and fibrogenic effects mediated by CB_1 receptors and CB_2 receptors respectively. Interestingly, there is evidence that the ECS is influenced by some dietary factors such as β-caryophyllene and particular flavonoids (see Sharma *et al.*, 2016). Thus, more experimental and clinical studies are needed to clarify the role of eCBs in different types of hypertension. Further therapeutic research should focus on the investigation of chronic effects of non-psychotic cannabinoids, such as CBD, peripherally restricted CB receptor ligands and their interaction with food or antihypertensive drugs targeting the RAS. This is important not only with respect to the search for new therapeutic strategies, but also in the context of cardiovascular effects of cannabinoids and the steadily increasing legalization of cannabis use for recreational and medical purposes.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.*, 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.*, 2017a,b,c).

Acknowledgement

The work was supported by the National Science Centre (Poland), research grant NCN 2012/05/B/NZ7/03102 and NCN 2015/19/B/NZ7/02270.

Conflict of interest

The authors declare no conflicts of interest.

References

Alexander SPH, Christopoulos A, Davenport AP, Kelly E, Marrion NV, Peters JA *et al.* (2017a). The Concise Guide to PHARMACOLOGY 2017/18: G protein-coupled receptors. Br J Pharmacol 174 (Suppl 1): S17–S129.

Alexander SPH, Fabbro D, Kelly E, Marrion NV, Peters JA, Faccenda E *et al.* (2017b). The Concise Guide to PHARMACOLOGY 2017/18: enzymes. Br J Pharmacol 174 (Suppl 1): S272–S359.

Alexander SP, Striessnig J, Kelly E, Marrion NV, Peters JA, Faccenda E *et al*. (2017c). The Concise Guide to PHARMACOLOGY 2017/18: voltage-gated ion channels. Br J Pharmacol 174 (Suppl 1): S160–S194.

Alshaarawy O, Elbaz HA (2016). Cannabis use and blood pressure levels: United States National Health and Nutrition Examination Survey, 2005–2012. J Hypertens 34: 1507–1512.

Bai Q, Zhang J, Zhang AH, Cheng LT, Duan JL, He L *et al*. (2012). Role of arachidonoylethanolamine in blood pressure regulation in volume-resistant patients on peritoneal dialysis. Int Urol Nephrol 44: 1855–1860.

Baldassarre M, Giannone FA, Napoli L, Tovoli A, Ricci CS, Tufoni M *et al*. (2013). The endocannabinoid system in advanced liver cirrhosis: pathophysiological implication and future perspectives. Liver Int 33: 1298–1308.

Baranowska-Kuczko M, Kloza M, Kozłowska H, Toczek M, Malinowska B (2017). Influence of cannabidiol on vascular function in hypertension. 8th European Workshop on Cannabinoid Research, The British Pharmacological Society. Abstract Booklet, 61.

Baranowska-Kuczko M, Kozłowska H, Kloza M, Karpińska O, Toczek M, Harasim E *et al*. (2016a). Protective role of cannabinoid CB_1 receptors and vascular effects of chronic administration of FAAH inhibitor URB597 in DOCA-salt hypertensive rats. Life Sci 151: 288–299.

Baranowska-Kuczko M, Kozłowska H, Kloza M, Karpińska O, Toczek M, Kozłowski M *et al*. (2016b). Cannabidiol-induced vasorelaxation in isolated human pulmonary and small mesenteric arteries of hypertensive rat – preliminary study. 26th Annual Symposium on Cannabinoids, International Cannabinoid Research Society. Book of abstracts, P1–19.

Bátkai S, Pacher P, Osei-Hyiaman D, Radaeva S, Liu J, Harvey-White J *et al*. (2004). Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. Circulation 110: 1996–2002.

Biernacki M, Ambrożewicz E, Gęgotek A, Toczek M, Bielawska K, Skrzydlewska E (2017). Redox system and phospholipid metabolism in the kidney of hypertensive rats after FAAH inhibitor URB597 administration. Redox Biol 15: 41–50.

Biernacki M, Łuczaj W, Gęgotek A, Toczek M, Bielawska K, Skrzydlewska E (2016). Crosstalk between liver antioxidant and the endocannabinoid systems after chronic administration of the FAAH inhibitor, URB597, to hypertensive rats. Toxicol Appl Pharmacol 301: 31–41.

Bonnet U (2016). Abrupt quitting of long-term heavy recreational cannabis use is not followed by significant changes in blood pressure and heart rate. Pharmacopsychiatry 49: 23–25.

Brozoski DT, Dean C, Hopp FA, Hillard CJ, Seagard JL (2009). Differential endocannabinoid regulation of baroreflex-evoked sympathoinhibition in normotensive versus hypertensive rats. Auton Neurosci 150: 82–93.

Burgueño AL, Sookoian S, Gianotti TF, Gemma C, Pirola CJ (2009). Genetic variation in the FAAH gene and metabolic syndrome-related phenotypes. Obesity (Silver Spring) 17: 1979–1980.

Caraceni P, Viola A, Piscitelli F, Giannone F, Berzigotti A, Cescon M *et al*. (2010). Circulating and hepatic endocannabinoids and endocannabinoid-related molecules in patients with cirrhosis. Liver Int 30: 816–825.

Cascio MG, Marini P (2015). Biosynthesis and fate of endocannabinoids. Handb Exp Pharmacol 231: 39–58.

Crawford WJ, Merritt JC (1979). Effects of tetrahydrocannabinol on arterial and intraocular hypertension. Int J Clin Pharmacol Biopharm 17: 191–196.

Domenicali M, Ros J, Fernández-Varo G, Cejudo-Martín P, Crespo M, Morales-Ruiz M *et al*. (2005). Increased anandamide induced relaxation in mesenteric arteries of cirrhotic rats: role of cannabinoid and vanilloid receptors. Gut 54: 522–527.

Engeli S, Blüher M, Jumpertz R, Wiesner T, Wirtz H, Bosse-Henck A *et al*. (2012). Circulating anandamide and blood pressure in patients with obstructive sleep apnea. J Hypertens 30: 2345–2351.

Gardiner SM, March JE, Kemp PA, Bennett T (2001). Regional haemodynamic responses to the cannabinoid agonist, WIN 55212-2, in conscious, normotensive rats, and in hypertensive, transgenic rats. Br J Pharmacol 133: 445–453.

Godlewski G, Alapafuja SO, Bátkai S, Nikas SP, Cinar R, Offertáler L *et al*. (2010). Inhibitor of fatty acid amide hydrolase normalizes cardiovascular function in hypertension without adverse metabolic effects. Chem Biol 17: 1256–1266.

Grzęda E, Schlicker E, Toczek M, Zalewska I, Baranowska-Kuczko M, Malinowska B (2017). CB_1 receptor activation in the rat paraventricular nucleus induces bi-directional cardiovascular effects via modification of glutamatergic and GABAergic neurotransmission. Naunyn Schmiedebergs Arch Pharmacol 390: 25–35.

Guo Z, Liu YX, Yuan F, Ma HJ, Maslov L, Zhang Y (2015). Enhanced vasorelaxation effect of endogenous anandamide on thoracic aorta in renal vascular hypertension rats. Clin Exp Pharmacol Physiol 42: 950–955.

Gyombolai P, Pap D, Turu G, Catt KJ, Bagdy G, Hunyady L (2012). Regulation of endocannabinoid release by G proteins: a paracrine mechanism of G protein-coupled receptor action. Mol Cell Endocrinol 353: 29–36.

Hall W (2015). What has research over the past two decades revealed about the adverse health effects of recreational cannabis use? Addiction 110: 19–35.

Harding SD, Sharman JL, Faccenda E, Southan C, Pawson AJ, Ireland S *et al*. (2018). The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. Nucl Acids Res 46: D1091–D1106.

Haspula D, Clark MA (2016). Heterologous regulation of the cannabinoid type 1 receptor by angiotensin II in astrocytes of spontaneously hypertensive rats. J Neurochem 139: 523–536.

Ho WS (2013). Modulation by 17β-estradiol of anandamide vasorelaxation in normotensive and hypertensive rats: a role for TRPV1 but not fatty acid amide hydrolase. Eur J Pharmacol 701: 49–56.

Ho WS, Gardiner SM (2009). Acute hypertension reveals depressor and vasodilator effects of cannabinoids in conscious rats. Br J Pharmacol 156: 94–104.

Ho WS, Hill MN, Miller GE, Gorzalka BB, Hillard CJ (2012). Serum contents of endocannabinoids are correlated with blood pressure in depressed women. Lipids Health Dis 11: 32.

Ho WSV, Kelly MEM (2017). Cannabinoids in the cardiovascular system. Adv Pharmacol 80: 329–366.

Hopps JJ, Dunn WR, Randall MD (2012). Enhanced vasorelaxant effects of the endocannabinoid-like mediator, oleamide, in hypertension. Eur J Pharmacol 684: 102–107.

Huang HC, Wang SS, Hsin IF, Chang CC, Lee FY, Lin HC *et al*. (2012). Cannabinoid receptor 2 agonist ameliorates mesenteric angiogenesis

and portosystemic collaterals in cirrhotic rats. Hepatology 56: 248–258.

Jadoon KA, Tan GD, O'Sullivan SE (2017). A single dose of cannabidiol reduces blood pressure in healthy volunteers in a randomized crossover study. JCI Insight 2. pii: 93760.

Karpińska O, Baranowska-Kuczko M, Kloza M, Ambrozewicz E, Kozłowski T, Kasacka I *et al*. (2017). Activation of CB_1 receptors by 2-arachidonoylglycerol attenuates vasoconstriction induced by U46619 and angiotensin II in human and rat pulmonary arteries. Am J Physiol Regul Integr Comp Physiol 312: R883–R893.

Kloza M, Baranowska-Kuczko M, Kozłowska H, Karpińska O, Harasim-Symbor E, Kasacka I *et al*. (2017a). Influence of the chronic administration of fatty acid amide hydrolase inhibitor, URB597, on the vascular reactivity in small mesenteric arteries of spontaneously hypertensive rats. J Physiol Pharmacol 68 (suppl. 1): 61.

Kloza M, Baranowska-Kuczko M, Malinowska B, Karpińska O, Harasim-Symbor E, Kasacka I *et al*. (2017b). The influence of DOCA-salt hypertension and chronic administration of the FAAH inhibitor URB597 on $KCa_{2.3}$/$KCa_{3.1}$-EDH-type relaxation in rat small mesenteric arteries. Vascul Pharmacol, pii: S1537-1891 (17)30198-2.

Lang H, Li Q, Yu H, Li P, Lu Z, Xiong S *et al*. (2015). Activation of TRPV1 attenuates high salt-induced cardiac hypertrophy through improvement of mitochondrial function. Br J Pharmacol 172: 5548–5558.

Li D, Chen BM, Peng J, Zhang YS, Li XH, Yuan Q *et al*. (2009). Role of anandamide transporter in regulating calcitonin gene-related peptide production and blood pressure in hypertension. J Hypertens 27: 1224–1232.

Li J, Kaminski NE, Wang DH (2003). Anandamide-induced depressor effect in spontaneously hypertensive rats: role of the vanilloid receptor. Hypertension 41: 757–762.

Malinowska B, Baranowska-Kuczko M, Schlicker E (2012). Triphasic blood pressure responses to cannabinoids: do we understand the mechanism? Br J Pharmacol 165: 2073–2088.

Malinowska B, Toczek M, Kossakowski R (2017). Function of presynaptic inhibitory cannabinoid CB_1 receptors on sympathetic nerves of spontaneously hypertensive rats. Naunyn Schmiedebergs Arch Pharmacol 390 (Suppl. 1): 36.

Marshall NJ, Liang L, Bodkin J, Dessapt-Baradez C, Nandi M, Collot-Teixeira S *et al*. (2013). A role for TRPV1 in influencing the onset of cardiovascular disease in obesity. Hypertension 61: 246–252.

Mattace Raso G, Pirozzi C, d'Emmanuele di Villa Bianca R, Simeoli R, Santoro A, Lama A *et al*. (2015). Palmitoylethanolamide treatment reduces blood pressure in spontaneously hypertensive rats: involvement of cytochrome p450-derived eicosanoids and renin angiotensin system. PLoS One 10: e0123602.

Murtaza G, Mermer P, Goldenberg A, Pfeil U, Paddenberg R, Weissmann N *et al*. (2017). TASK-1 potassium channel is not critically involved in mediating hypoxic pulmonary vasoconstriction of murine intra-pulmonary arteries. PLoS One 12: e0174071.

Mutombo PB, Yamasaki M, Nabika T, Shiwaku K (2012). Cannabinoid receptor 1 (CNR1) 4895 C/T genetic polymorphism was associated with obesity in Japanese men. J Atheroscler Thromb 19: 779–785.

Nahas GG, Schwartz IW, Adamec J, Manger WM (1973). Tolerance of delta-9-tetrahydrocannabinol in the spontaneously hypertensive rat. Proc Soc Exp Biol Med 142: 58–60.

Orsini J, Blaak C, Rajayer S, Gurung V, Tam E, Morante J *et al*. (2016). Prolonged cardiac arrest complicating a massive ST-segment elevation myocardial infarction associated with marijuana consumption. J Community Hosp Intern Med Perspect 6: 31695.

O'Sullivan SE (2015). Endocannabinoids and the cardiovascular system in health and disease. Handb Exp Pharmacol 231: 393–422.

O'Sullivan SE, Randall MD, Gardiner SM (2007). The *in vitro* and *in vivo* cardiovascular effects of Δ^9-tetrahydrocannabinol in rats made hypertensive by chronic inhibition of nitric-oxide synthase. J Pharmacol Exp Ther 321: 663–672.

Pędzińska-Betiuk A, Weresa J, Toczek M, Baranowska-Kuczko M, Kasacka I, Harasim-Symbor E *et al*. (2017). Chronic inhibition of fatty acid amide hydrolase by URB597 produces differential effects on cardiac performance in normotensive and hypertensive rats. Br J Pharmacol 174: 2114–2129.

Pertwee RG (2015). Endocannabinoids and their pharmacological actions. Handb Exp Pharmacol 231: 1–37.

Polak A, Harasim-Symbor E, Malinowska B, Chabowski A (2017a). Effects of endocannabinoid system activation on myocardial glucose metabolism in rat model of secondary hypertension. J Physiol Pharmacol 68 (suppl. 1): 65.

Polak A, Harasim-Symbor E, Malinowska B, Kasacka I, Pędzińska-Betiuk A, Weresa J *et al*. (2017b). The effects of chronic FAAH inhibition on myocardial lipid metabolism in normotensive and DOCA-salt hypertensive rats. Life Sci 183: 1–10.

Pulgar VM, Yamaleyeva LM, Varagic J, McGee CM, Bader M, Dechend R *et al*. (2014). Increased angiotensin II contraction of the uterine artery at early gestation in a transgenic model of hypertensive pregnancy is reduced by inhibition of endocannabinoid hydrolysis. Hypertension 64: 619–625.

Reichenbach V, Ros J, Fernández-Varo G, Casals G, Melgar-Lesmes P, Campos T*et al*. (2012). Prevention of fibrosis progression in CCl_4-treated rats: role of the hepatic endocannabinoid and apelin systems. J Pharmacol Exp Ther 340: 629–637.

Reinhard W, Stark K, Neureuther K, Sedlacek K, Fischer M, Baessler A *et al*. (2008). Common polymorphisms in the cannabinoid CB2 receptor gene (CNR2) are not associated with myocardial infarction and cardiovascular risk factors. Int J Mol Med 22: 165–174.

Ruilope LM, Després JP, Scheen A, Pi-Sunyer X, Mancia G, Zanchetti A *et al*. (2008). Effect of rimonabant on blood pressure in overweight/obese patients with/without co-morbidities: analysis of pooled RIO study results. J Hypertens 26: 357–367.

Sarzani R, Bordicchia M, Salvi F, Cola G, Franchi E, Battistoni I *et al*. (2008). A human fatty acid amide hydrolase (FAAH) functional gene variant is associated with lower blood pressure in young males. Am J Hypertens 21: 960–963.

Schaich CL, Grabenauer M, Thomas BF, Shaltout HA, Gallagher PE, Howlett AC *et al*. (2016). Medullary endocannabinoids contribute to the differential resting baroreflex sensitivity in rats with altered brain renin–angiotensin system expression. Front Physiol 7: 207.

Schaich CL, Shaltout HA, Brosnihan KB, Howlett AC, Diz DI (2014). Acute and chronic systemic CB_1 cannabinoid receptor blockade improves blood pressure regulation and metabolic profile in hypertensive (mRen2)27 rats. Physiol Rep 2 pii: e12108.

Sharma C, Al Kaabi JM, Nurulain SM, Goyal SN, Kamal MA, Ojha S (2016). Polypharmacological properties and therapeutic potential of β-caryophyllene: a dietary phytocannabinoid of pharmaceutical promise. Curr Pharm Des 22: 3237–3264.

Shi RZ, Hu CP, Luo D, Li D, Pan W, Li SX *et al*. (2012). Decreased anandamide transporter activity and calcitonin gene-related peptide

production in spontaneously hypertensive rats: role of angiotensin II. Eur J Pharmacol 680: 81–87.

Stanley C, O'Sullivan SE (2014). Vascular targets for cannabinoids: animal and human studies. Br J Pharmacol 171: 1361–1378.

Toczek M, Baranowska-Kuczko M, Grzęda E, Pędzińska-Betiuk A, Weresa J, Malinowska B (2016). Age-specific influences of chronic administration of the fatty acid amide hydrolase inhibitor URB597 on cardiovascular parameters and organ hypertrophy in DOCA-salt hypertensive rats. Pharmacol Rep 68: 363–369.

Toczek M, Schlicker E, Grzęda E, Malinowska B (2015). Enhanced function of inhibitory presynaptic cannabinoid CB_1 receptors on sympathetic nerves of DOCA-salt hypertensive rats. Life Sci 138: 78–85.

Van Gaal LF, Scheen AJ, Rissanen AM, Rössner S, Hanotin C, Ziegler O *et al*. (2008). Long-term effect of CB_1 blockade with rimonabant on cardiometabolic risk factors: two year results from the RIO-Europe Study. Eur Heart J 29: 1761–1771.

Vandrey R, Umbricht A, Strain EC (2011). Increased blood pressure after abrupt cessation of daily cannabis use. J Addict Med 5: 16–20.

Varma DR, Goldbaum D (1975). Effect of delta9-tetrahydrocannabinol on experimental hypertension in rats. J Pharm Pharmacol 27: 790–791.

Wahn H, Wolf J, Kram F, Frantz S, Wagner JA (2005). The endocannabinoid arachidonyl ethanolamide (anandamide) increases pulmonary arterial pressure via cyclooxygenase-2 products in isolated rabbit lungs. Am J Physiol Heart Circ Physiol 289: H2491–H2496.

Wang Y, Kaminski NE, Wang DH (2007). Endocannabinoid regulates blood pressure via activation of the transient receptor potential vanilloid type 1 in Wistar rats fed a high-salt diet. J Pharmacol Exp Ther 321: 763–769.

Wang T, Li GQ, Zhang HP, Zhang Y, Li Q (2017). Overactivation of cannabinoid receptor type 1 in rostral ventrolateral medulla promotes cardiovascular responses in spontaneously hypertensive rats. J Hypertens 35: 538–545.

Wenzel D, Matthey M, Bindila L, Lerner R, Lutz B, Zimmer A *et al*. (2013). Endocannabinoid anandamide mediates hypoxic pulmonary vasoconstriction. Proc Natl Acad Sci 110: 18710–18715.

Wheal AJ, Bennett T, Randall MD, Gardiner SM (2007a). Cardiovascular effects of cannabinoids in conscious spontaneously hypertensive rats. Br J Pharmacol 152: 717–724.

Wheal AJ, Bennett T, Randall MD, Gardiner SM (2007b). Effects of chronic nitric oxide synthase inhibition on the cardiovascular responses to cannabinoids *in vivo* and *in vitro*. Br J Pharmacol 150: 662–671.

Wheal AJ, Randall MD (2009). Effects of hypertension on vasorelaxation to endocannabinoids *in vitro*. Eur J Pharmacol 603: 79–85.

Yang YY, Lin HC (2015). Alteration of intrahepatic microcirculation in cirrhotic livers. J Chin Med Assoc 78: 430–437.

Yang D, Luo Z, Ma S, Wong WT, Ma L, Zhong J *et al*. (2010). Activation of TRPV1 by dietary capsaicin improves endothelium-dependent vasorelaxation and prevents hypertension. Cell Metab 12: 130–141.

Zygmunt PM, Chuang H, Movahed P, Julius D, Högestätt ED (2000). The anandamide transport inhibitor AM404 activates vanilloid receptors. Eur J Pharmacol 396: 39–42.