British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2011.01747.x
www.brjpharmacol.org

REVIEW

Triphasic blood pressure responses to cannabinoids: do we understand the mechanism?

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Keywords
blood pressure response; anandamide; Δ^9-tetrahydrocannabinol; WIN55212-2; anaesthetized rodents; conscious rat; humans; cannabinoid CB_1 receptor; vanilloid TRPV1 receptor; endothelial cannabinoid receptor

Received
19 July 2011
Revised
29 September 2011
Accepted
7 October 2011

The cannabinoids comprise three major classes of substances, including compounds derived from the cannabis plant (e.g. Δ^9-tetrahydrocannabinol and the chemically related substances CP55940 and HU210), endogenously formed (e.g. anandamide) and synthetic compounds (e.g. WIN55212-2). Beyond their psychotropic effects, cannabinoids have complex effects on blood pressure, including biphasic changes of Δ^9-tetrahydrocannabinol and WIN55212-2 and an even triphasic effect of anandamide. The differing pattern of blood pressure changes displayed by the three types of compounds is not really surprising since, although they share an agonistic effect at cannabinoid CB_1 and CB_2 receptors, some compounds have additional effects. In particular, anandamide is known for its pleiotropic effects, and there is overwhelming evidence that anandamide influences blood pressure via (i) CB_1 receptors, (ii) TRPV1 receptors, (iii) endothelial cannabinoid receptors and (iv) degradation products. This review is dedicated to the description of the effects of externally added cannabinoids on cardiovascular parameters *in vivo*. First, the cardiovascular effects of cannabinoids in anaesthetized animals will be highlighted since most data have been generated in experiments of that type. The text will follow the three phases of anandamide on blood pressure, and we will check to which extent cardiovascular changes elicited by other cannabinoids show overlap with those effects or differ. The second part will be dedicated to the cardiovascular effects of the cannabinoids in conscious animals. In the third part, cardiovascular effects in humans will be discussed, and similarities and differences with respect to the data from animals will be examined.

Abbreviations

2-AG, 2-arachidonoyl glycerol; AEA, anandamide; AM251, (*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide); AM404, *N*-(4-hydroxyphenyl)-5*Z*,8*Z*, 11*Z*,14*Z*-eicosatetraenamide; AM3506, (5-[4-hydroxyphenyl] pentanesulphonyl fluoride); BP, blood pressure; CGRP, calcitonin-gene-related-peptide; CP55940, (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)-cyclohexanol; dPAG, dorsal periaqueductal gray; FAAH, fatty acid amide hydrolase; HR, heart rate; HU210, (6aR)-*trans*-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6, 6-dimethyl-6*H*-dibenzo[b,d]pyran-9-methanol; i.c.; into the cisterna magna; ICI 118551, (erythro-(+/−)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol); i.t.; intrathecal; MAGL, monoacylglycerole lipase; MethAEA, R-(+)-methanandamide; MK-801, ((5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo(a,d)-cyclohepten-5,10-imine hydrogen maleate); NADA, N-arachidonoyl-dopamine; NAT, neuronal noradrenaline transporter; NTS, nucleus tractus solitarii; O-1918 (1,3-dimethoxy-5-methyl-2-[(1*R*,6*R*)-3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-benzene); PAG, periaqueductal gray; PVN, paraventricular nucleus of the hypothalamus; RSNA, renal sympathetic nerve activity; RVLM, rostral ventrolateral medulla; SHR, spontaneously hypertensive rats; Δ^9-THC, Δ^9-tetrahydrocannabinol; TRPV1, transient receptor potential channels of V1 type; URB597, (3′-(aminocarbonyl)[1,1′-biphenyl]-3-yl)-cyclohexylcarbamate); WIN55212-2, (*R*(+) [2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)-methanone mesylate)

Introduction

Cannabis preparations have been used for recreational and therapeutic purposes for thousands of years, but details of the mechanisms of action have been disclosed during the recent 20 years only. Cannabis preparations or, stricto sensu, some of their constituents activate an endogenous system of the body, the so-called endocannabinoid system, which is activated also by endogenously formed compounds, the so-called endocannabinoids that chemically differ from the plant-derived cannabinoids. The endocannabinoid system plays a role under physiological and many pathophysiological conditions not only in the brain but also in peripheral tissues. Consequently, it has not only to do with psychotropic but also with many other effects including cardiovascular function. Thus, endocannabinoids are implicated in the pathogenesis of hypertension, hypotension associated with haemorrhagic, endotoxic and cardiogenic shock and with advanced liver cirrhosis or in the control of atherosclerosis (for review, see Pacher *et al.*, 2005; Malinowska *et al.*, 2008; Bátkai and Pacher, 2009). The possibility has even been considered that diseases of the cardiovascular system might be treated by targeting the endocannabinoid system (Pacher *et al.*, 2006).

In the present review, the effects of several types of cannabinoids on cardiovascular parameters *in vivo* will be described. The review is dedicated to the description of haemodynamic effects of externally added cannabinoids, and so the involvement of the endocannabinoid system in the pathogenesis of many diseases of the cardiovascular system will not be touched here. However, the effects of cannabinoids in animals or humans with hypertension will be covered. First, the cardiovascular effects of cannabinoids in anaesthetized animals will be highlighted since most data have been generated in experiments of that type. The text will follow the three phases of the endocannabinoid arachidonoylethanolamide (anandamide, AEA; for chemical structure, see Figure 1) on blood pressure (BP) in anaesthetized rodents (e.g. Varga *et al.*, 1995; Malinowska *et al.*, 2001a; Pacher *et al.*, 2004), and we will check to which extent cardiovascular effects elicited by other cannabinoids show overlap with those effects or differ. The second part will be dedicated to the cardiovascular effects of the cannabinoids in conscious animals. In the third part, cardiovascular effects in humans will be discussed, and similarities and differences with respect to the data from animals will be examined. The review will be preceded by a very brief overview about the cannabinoids and the major constituents of the endocannabinoid system. For detailed information related to the latter two topics, the reader is referred to excellent reviews; only some of them have been quoted in the subsequent two paragraphs. Drug/molecular target nomenclature conforms to the British Journal of Pharmacology's *Guide to Receptors and Channels* (Alexander *et al.*, 2011).

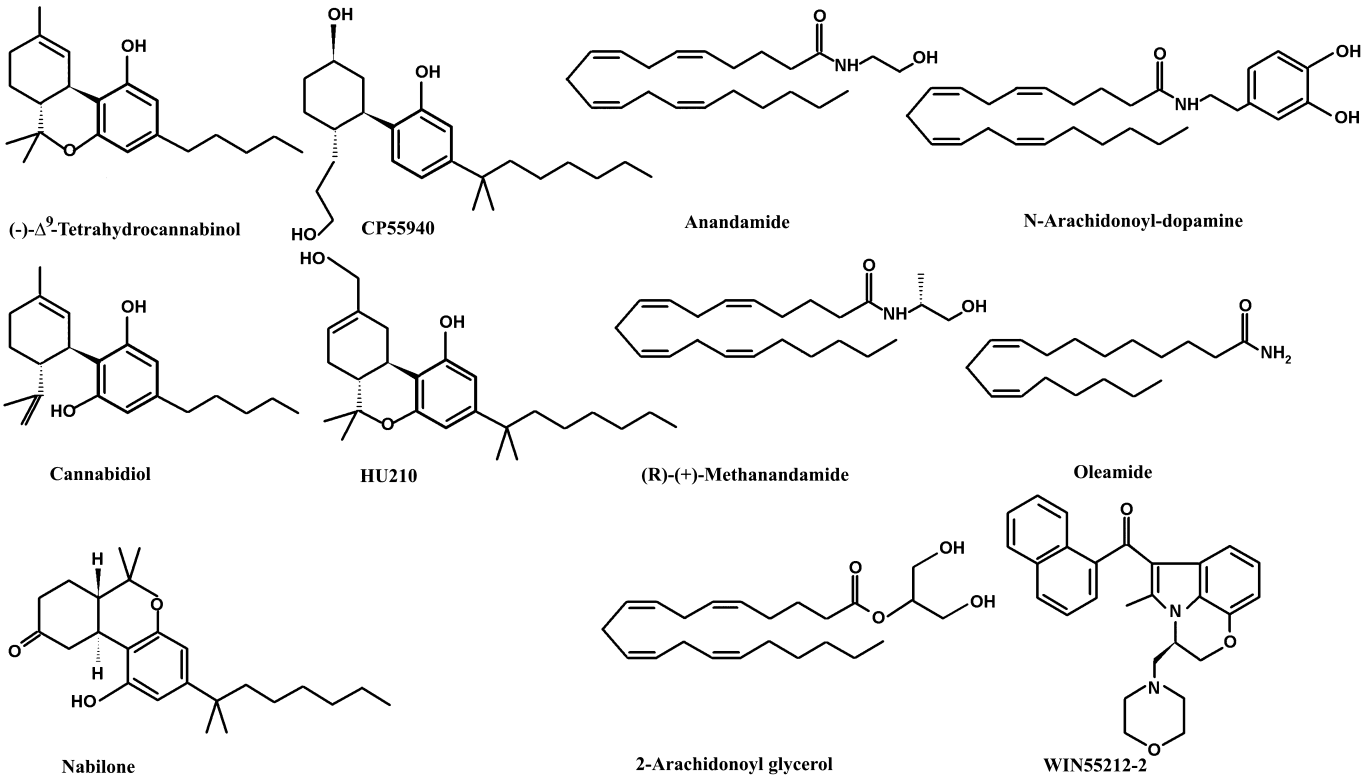

Figure 1

Chemical structures of cannabinoids.

The term 'cannabinoids', which originally referred to a series of chemically closely related compounds occurring in the cannabis plant *Cannabis sativa*, var. *indica*, is now used rather in a functional sense and comprises substances of different chemical structure activating the endocannabinoid system (Pertwee, 2005; Figure 1). In the class of compounds isolated from the cannabis plant, Δ^9-tetrahydrocannabinol (Δ^9-THC) is the most important one. HU210 and CP55940, chemically modified from Δ^9-THC, may also be entered into this class; they do not play a role in humans but are frequently used for scientific purposes (Pertwee, 2005). Cannabidiol, which occurs in the cannabis plant in a similarly high concentration as Δ^9-THC, is devoid of psychotropic effects (Russo and Guy, 2006; Izzo *et al.*, 2009). The second class of cannabinoids are the endogenously formed endocannabinoids, that is derivatives from arachidonic acid synthetized by the cell membrane on demand. The aforementioned AEA and 2-arachidonoyl glycerol (2-AG) are the two compounds that have so far attracted most attention (Di Marzo *et al.*, 2005; Howlett *et al.*, 2011). WIN55212-2 belongs to a third class of cannabinoids, which are chemically unrelated to either of the aforementioned classes; the compound is potent and less lipophilic compared with the above compounds and is frequently used for scientific purposes (Pertwee, 2005).

The major molecular targets for the effect of the cannabinoids as far as they play a role for the cardiovascular system are (i) cannabinoid receptors (primarily CB_1), (ii) the vanilloid transient receptor potential (TRP) channels of V1 type (TRPV1) receptor and (iii) the so-called endothelial cannabinoid receptor. Only AEA activates each of those entities, whereas the other cannabinoids activate only part of them (Pertwee *et al.*, 2010). The CB_1 receptor, which is $G_{i/o}$-protein-coupled and serves as a presynaptic inhibitory receptor, occurs in very high density in the brain and is the substrate of the psychotropic effects of cannabinoids; it is expressed in low density also in peripheral tissues (Pertwee, 2005; Pacher *et al.*, 2006). Unlike the CB_1 receptor, the TRPV1 receptor is a ligand-gated ion channel usually activated by extreme physical alterations (e.g. low pH value or high temperature) but also by high concentrations of AEA (Gunthorpe *et al.*, 2002). The molecular properties of the endothelial cannabinoid receptor, sometimes and herein termed CB_x receptor, have not yet been disclosed; its activation leads to dilatation of some vessels, e.g. of the mesenterial arterial bed (Offertáler *et al.*, 2003; Kozłowska *et al.*, 2007, 2008). Moreover, in some instances, the endocannabinoids act indirectly via their degradation products (Kozłowska *et al.*, 2008 and references therein). AEA and 2-AG are degraded to arachidonic acid mainly by fatty acid amide hydrolase (FAAH) and monoacylglycerole lipase (MAGL), respectively, and new compounds (e.g. prostanoids) can be formed from arachidonic acid. Investigation of the effects mediated via the aforementioned receptors is facilitated by the availability of CB_1 (Valverde *et al.*, 2005) and TRPV1 receptor knockout mice (Caterina *et al.*, 2000). Moreover, antagonists are available comprising rimonabant and AM251 for the CB_1 (Pertwee, 2005), capsazepine for the TRPV1 (Gunthorpe *et al.*, 2002) and O-1918 for the CB_x receptor (Offertáler *et al.*, 2003). The FAAH inhibitor URB597 has to be mentioned here as well (Kathuria *et al.*, 2003).

Influence of cannabinoids on cardiovascular parameters in anaesthetized animals

In rats anaesthetized with urethane (Varga *et al.*, 1995; Lake *et al.*, 1997a,b; Malinowska *et al.*, 2001a, 2010; Kwolek *et al.*, 2005; Zakrzeska *et al.*, 2010) or in mice anaesthetized with pentobarbitone (Pacher *et al.*, 2004, 2005) rapid i.v. injection of AEA (1–57 μmol·kg^{-1}) induces typical triphasic changes in cardiovascular parameters (Figure 2; Table 1). The initial phase I is characterized by a rapid and pronounced bradycardia and a transient drop in BP associated with a decrease in cardiac contractility and an increase in total peripheral resistance. The subsequent phase II consists of a brief pressor response (lasting for about 30–60 s) associated with an increase in cardiac contractility and blood flow in the mesenteric and renal vascular beds. In the final phase III, a more

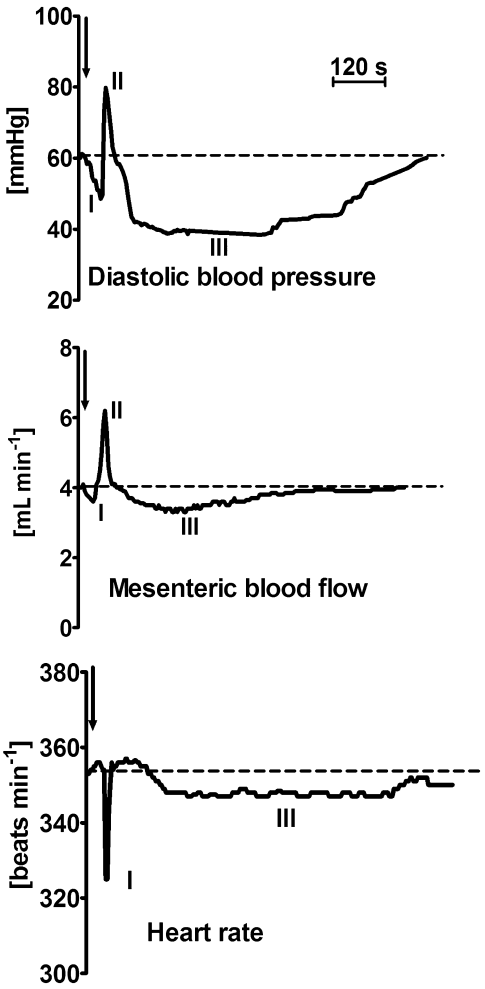

Figure 2

Typical traces showing the changes in diastolic blood pressure, mesenteric blood flow and heart rate induced by i.v. injection of anandamide (3 μmol·kg^{-1}) in a rat anaesthetized with urethane. Arrows indicate drug application. From Zakrzeska *et al.* (2010) (modified).

Table 1

Influence of cannabinoids on selected parameters of the animal cardiovascular system

Species	Agonist	Doses ($\mu mol \cdot kg^{-1}$)	Route of administration	Anaesthesia	Effects	Examples of the literature
Rat	AEA	0.9–58	i.v.	urethane	I: ↓BP, ↓HR; II: ↑BP; III: ↓BP, ↓HR See Figure 1	Lake *et al.* (1997a); Malinowska *et al.* (2001a)
Mouse	AEA	58	i.v.	pentobarbital	I: ↓BP, ↓HR; ↓cardiac contractility; ↑TPR; II: ↑BP; ↑cardiac contractility; III: ↓BP, ↓HR, ↓cardiac contractility; ↓TPR	Pacher *et al.* (2004)
Rat	MethAEA	0.03–3	i.v.	urethane	I: ↓BP, ↓HR; II: ↑BP; III: ↓BP, ↓HR	Malinowska *et al.* (2001a)
Rat	THC	0.03–32	i.v.	urethane	II: ↑BP; III: ↓BP, ↓HR	Lake *et al.* (1997a)
Rat	WIN	0.02–19	i.v.	urethane	↓BP, ↓HR, ↓[NAdr]	Lake *et al.* (1997a); Niederhoffer *et al.* (2003)
	CP	0.003–2.7			↓BP, ↓HR, ↓[NAdr]	
	HU210	0.0008–0.8			↓BP, ↓HR, ↓[NAdr]	
Rat	HU210	0.03–2.6	i.v.	pentobarbital	↓BP, ↓HR	Vidrio *et al.* (1996)
Rat	2-AG	2.6; 7.9	i.v.	urethane	↓BP, ↑HR	Varga *et al.* (1998)
Mouse	2-AG	26	i.v.	pentobarbital	↓BP, ↑HR	Járai *et al.* (2000)
Rat	NADA	2.3–23	i.v.	conscious	I. ↓BP, II. ↑BP, III. ↓BP, ↑HR	Wang and Wang (2007)
Rat	AEA	0.2–8.6	i.v.	conscious	↑BP, ↓HR, vasoconstriction in renal and mesenteric and vasodilatation in hindquarters vascular bed	Gardiner *et al.* (2002a, 2009)
	MethAEA	8.3				
Rat	WIN	0.3	i.v.	conscious	↑BP, ↓HR, vasoconstriction in renal and mesenteric and vasodilatation in hindquarters vascular bed	Gardiner *et al.* (2002b); O'Sullivan *et al.* (2007)
	HU210	0.3				
	THC	3.2				
Monkey	THC	1.6	i.v.	conscious	↑HR, ↓BP	Fredericks *et al.* (1981)
Rat	oleamide	35 and 71	i.p.	conscious	No changes in BP and HR	Huitrón-Reséndiz *et al.* (2001)
Rat	THC	1–95	i.a.	urethane	↑perfusion pressure in the hindquarters	Adams *et al.* (1976)
Rat	AEA	86–863*	i.a.	urethane	↓BP, ↑ventilation	Smith and McQueen (2001)
Rat	AEA	2900*	i.a.	decerebrate	↑BP	Williams *et al.* (2008)
Rat	AEA	50 and 100*	i.t.	urethane	↓BP	García *et al.* (2003; 2009)
	MethAEA	50 and 100*			↓BP	
	WIN	20*			↓BP	
	NADA	50 and 100*			↓BP	

Table 1

Continued

Species	Agonist	Doses ($\mu mol \cdot kg^{-1}$)	Route of administration	Anaesthesia	Effects	Examples of the literature
Rat	AEA	30–100*	i.c.v.	urethane	↑BP but only in the presence of AM251 and ruthenium red	Malinowska *et al.* (2010)
Rat	WIN	0.06*	i.c.	urethane	↑BP, ↑[NAdr], ↓HR	Pfitzer *et al.* (2004)
Rabbit	WIN	0.002 and 0.020*	i.c.	conscious	↑RSNA, ↑[NAdr], ↑BP (only the highest doses) and ↓HR	Niederhoffer and Szabo (2000)
	CP	0.003 and 0.026*				
Rat	AEA	0.0017*	PAG	pentobarbital	↑BP, ↑RSNA	Dean (2011)
Rat	CBD	60*	BNST	conscious	↑baroreflex-induced bradycardia	Alves *et al.* (2010)
Rat	WIN	1.9 and 9.6*	NTS	urethane	No changes in BP, HR and [NAdr]	Niederhoffer *et al.* (2003)
Dog	WIN	0.00125–0.0015*	NTS	α-chloralose + urethane	No changes in BP and baroreflex sensitivity	Rademacher *et al.* (2003)
Rat	AEA	0.0035*	NTS	pentobarbital	prolongation of baroreflex inhibition of RSNA	Brozoski *et al.* (2009)
Rat	AEA	11.5	i.v.	urethane	↑activity of barosensitive neurons in the RVLM	Varga *et al.* (1996)
Rat	WIN	1.9 and 9.6*	RVLM	urethane	↓BP (slight), ↓HR (slight), no changes in [NAdr]	Niederhoffer *et al.* (2003)
Rat	WIN	0.00005–0.0005*	RVLM	urethane	↑RSNA, ↑BP; no changes in HR	Padley *et al.* (2003)
	HU210	0.0005*			↑RSNA, ↑BP; no changes in HR	

*nmol per rat.

BNST, bed nucleus of the stria terminalis; CBD, cannabidiol; CP, CP55940; [NAdr], plasma noradrenaline concentration; TPR, total peripheral resistance; WIN, WIN55212-2; I–III, phases of cardiovascular changes. Since doses have been given in mg or μg rather than in μmol in many papers, we re-calculated them in μmol or nmol (molecular weights: AEA – 347.5; 2-AG – 378.6; CP – 376.6; HU210 – 386.6; MethAEA – 361.6; NADA – 439.6; oleamide – 281.5; THC – 314.4; WIN – 522.6).

prolonged (lasting up to 10 min), marked decrease in BP accompanied by a fall in mesenteric and renal blood flow, a marked decrease in cardiac contractility and a slight decrease in heart rate (HR) and total peripheral resistance occurs.

Similar triphasic changes in cardiovascular parameters were also obtained after i.v. administration of methanandamide (MethAEA; a stable analogue of anandamide; for chemical structure, see Figure 1), arguing against the possibility that AEA acts indirectly via its arachidonic acid metabolites (Malinowska *et al.*, 2001a). A triphasic response occurred also after administration of the endovanilloid N-arachidonoyldopamine (NADA; for chemical structure, see Figure 1) with the difference that in phase III the decrease in BP was associated with a pronounced increase in HR (Wang and Wang, 2007). Δ^9-THC elicited biphasic (phases II and III only) and 2-AG, HU210, CP55940 and WIN55212-2 elicited monophasic cardiovascular responses (phase III only) (Vidrio *et al.*, 1996; Lake *et al.*, 1997a; Niederhoffer *et al.*, 2003; Table 1). The decrease in BP obtained with 2-AG, unlike that obtained with the other three cannabinoids, was associated with an increase in HR (Table 1).

Phase I

The first phase of cardiovascular changes induced by AEA in anaesthetized rodents is mainly a short-lasting bradycardia and hypotension. The following observations clearly prove that this phase is mediated by vanilloid TRPV1 receptors: (i) It was absent in TRPV1$^{-/-}$ mice (Pacher *et al.*, 2004); (ii) it was induced not only by rapid injection of AEA and MethAEA but also by the TRPV1 receptor agonist capsaicin with the following rank order of potencies: capsaicin > MethAEA > AEA (the apparent ED_{50} that decreased HR by 150 beats·min^{-1} were 0.013, 0.744 and 7.562 μmol·kg^{-1}, respectively); and (iii) it was blocked by a selective (capsazepine) and non-selective (ruthenium red) TRPV1 receptor antagonist but (iv) not by the CB_1 receptor antagonist rimonabant (Smith and McQueen, 2001; Malinowska *et al.*, 2001a; Pacher *et al.*, 2004). The profound drop in cardiac contractility that accompanied the reflex bradycardia and hypotension was also absent in TRPV1$^{-/-}$ mice (Pacher *et al.*, 2004).

Phase I involves the so-called Bezold–Jarisch reflex, which leads to bradycardia plus hypotension (Campagna and Carter, 2003), and can also be induced by the activation of serotonin 5-HT_3 receptors, which like the TRPV1 receptors are located on sensory vagal nerves in the heart (Figure 3). This reflex is blocked by atropine, bilateral vagotomy (Varga *et al.*, 1995) and is absent in pithed rats; the latter model offers the opportunity to study drug effects on the peripheral cardiovascular system only (Kwolek *et al.*, 2005; Zakrzeska *et al.*, 2010). Phase I is the most homogeneous and the best explained change. However, it is detected relatively rarely, probably because of two reasons. First, this short-lasting phase is induced by relatively high doses of the agonist; for the apparent ED_{50} value see above. Such responses can be achieved only by rapid i.v. injection of the agonist (Pacher *et al.*, 2005; also pers. obs.). Second, reflex responses are diminished by pentobarbitone anaesthesia (for literature, see Malinowska *et al.*, 2001a). This explains why phase I occurred in rats anaesthetized with urethane but could be elicited not at all (Kwolek *et al.*, 2005) or only at a high dose of 57 μmol·kg^{-1} in rats treated with pentobarbitone instead (Pacher *et al.*, 2004, 2005). Phase I is not induced by most of the other cannabinoids including CP55940 and WIN55212-2 since they do not possess affinity towards TRPV1 receptors. The latter two compounds are even known to inhibit the afferent limb of the 5-HT_3 receptor-mediated Bezold–Jarisch reflex (Godlewski *et al.*, 2003).

It has been postulated that TRPV1 receptors located on cardiac afferents may serve also as a molecular detector of myocardial ischemia to activate cardiac nociceptors (Pan and Chen, 2004) and a sympathoexcitatory reflex (Zahner *et al.*, 2003). We found (Łupiński *et al.*, 2011) that acute myocardial ischemia in rats increased by about 100% the reflex bradycardia elicited (i) by AEA (in a manner sensitive to the TRPV1 receptor antagonist capsazepine) and (ii) the 5-HT_3 receptor agonist phenylbiguanide (in a manner sensitive to the 5-HT_3 receptor antagonist ondansetron). Moreover, the blockade of TRPV1 receptors increased the ischemic area size and decreased the survival rate after acute myocardial ischaemia. A beneficial cardioprotective influence of these receptors against cardiac injury has been demonstrated in the isolated heart. Moreover, TRPV1 gene deletion increased the mortality of mice 3 days after myocardial infarction (Huang *et al.*, 2009).

Phase II

Phase II of the AEA-induced changes in the cardiovascular parameters of anaesthetized rodents consists of a brief pressor effect. The highest doses of AEA under study (30 and 60 μmol·kg^{-1}) in anaesthetized rats and mice increased BP by about 60% and even 120% of the basal values respectively. The lack of the ability of some CB_1 receptor agonists (e.g. WIN55212-2 or CP55940) to induce this phase and the lack of effect of rimonabant on the AEA-stimulated pressor effect argue against the involvement of CB_1 receptors (Varga *et al.*, 1995; Lake *et al.*, 1997a,b; Malinowska *et al.*, 2001a; Pacher *et al.*, 2004; Kwolek *et al.*, 2005). Unfortunately, the pressor response to AEA has not been analysed in $CB_1^{-/-}$ mice (Járai *et al.*, 1999; Ledent *et al.*, 1999).

The most trivial hypothesis, that is that the pressor effect of AEA can be the simple response to the preceding hypotension (phase I), was excluded because it was not modified by acute surgical transection of the cervical spinal cord, atropine pretreatment (Varga *et al.*, 1995), bilateral vagotomy or pithing (Kwolek *et al.*, 2005), that is measures that inhibit or abolish the reflex loop of the Bezold-Jarisch reflex. In pithed rats, the pressor effect of the highest dose of the endocannabinoid (30 μmol·kg^{-1}) was even higher than in animals with intact spinal cord, suggesting the participation of peripheral mechanism(s). However, the possibility has to be considered that the particularly marked increase in BP is related to an inhibitory effect of pithing on the subsequent depressor phase III.

Phase II may be, at least partially, due to the activation of vanilloid TRPV1 receptors since it was reduced in TRPV1$^{-/-}$ pentobarbital-anaesthetized mice (Pacher *et al.*, 2004; in this case, anandamide at a high dose of 57 μmol·kg^{-1} was used). Moreover, in urethane-anesthetized rats, it was induced not only by AEA and MethAEA but also by capsaicin, and it was inhibited by a non-selective antagonist of TRPV1 receptors, ruthenium red (Malinowska *et al.*, 2001a; Pacher *et al.*, 2004; Kwolek *et al.*, 2005). Surprisingly, capsazepine, a selective

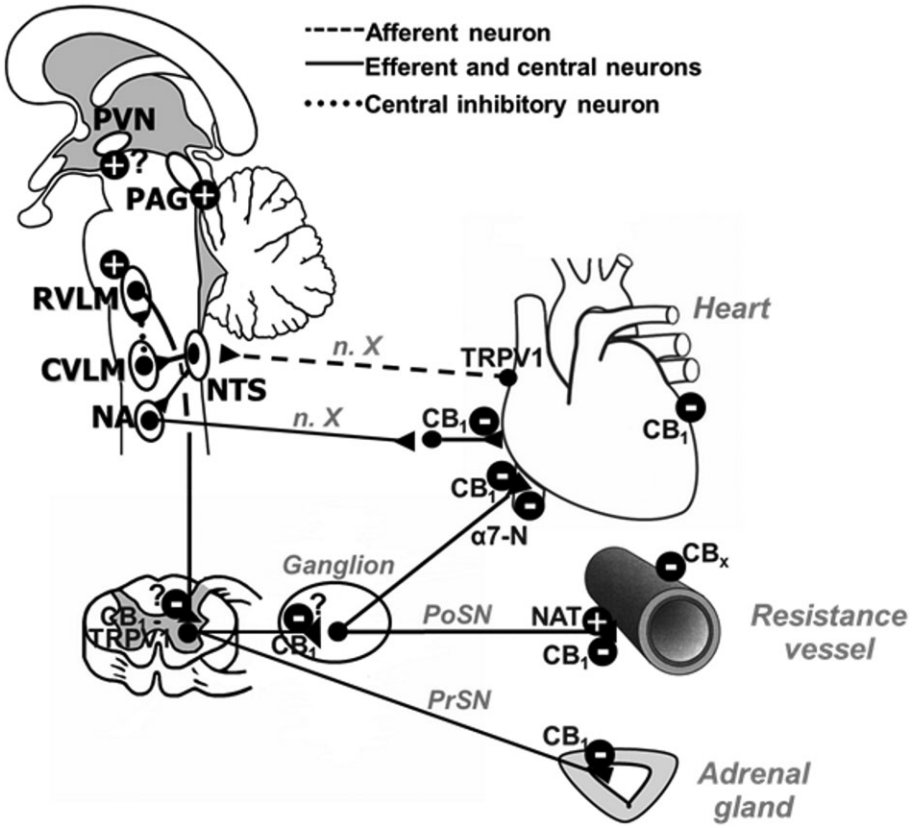

Figure 3

Potential central and peripheral mechanisms involved in the cardiovascular effects of cannabinoids. Plus and minus signs in the CNS mean stimulatory and inhibitory effects on cardiovascular parameters respectively. In the periphery, minus signs mean inhibition of transmitter release (where applicable) or decrease in the tone of heart or vascular muscle; the plus sign means stimulation of the carrier-mediated noradrenaline release. CVLM; caudal ventrolateral medulla; n. X, vagal nerve; NA, nucleus ambiguus; PoSN, postganglionic sympathetic nerve; PrSN, preganglionic sympathetic nerve. Receptors: α7-nicotinic acetylcholine receptor (α7-N), cannabinoid CB_1, CB_2 and CB_x receptors and vanilloid TRPV1 receptor.

antagonist of these receptors, failed to modify the AEA-stimulated increase in BP (Kwolek *et al.*, 2005). This may result from species differences between mouse and rat or the involvement of another TRPV receptor subtype in the rat (Alexander *et al.*, 2011). In this context, the obvious discrepancy has to be discussed that the TRPV1 (or a closely related) receptor is capable of inducing both phases I and II, which extremely differ with respect to their influence on cardiovascular parameters. A solution of this discrepancy may be that TRPV receptors do not only elicit the Bezold–Jarisch reflex (phase I) but also lead to a sympathoexcitatory response with a brief increase in BP. Such a sympathoexcitatory reflex response was induced by the activation of TRPV1 receptors located on afferent nerve fibres in the rat heart (Zahner *et al.*, 2003).

There may be additional components contributing to phase II. Thus, we found that the L-type calcium channel blocker nifedipine reduced this phase both in intact urethane-anaesthetized and pithed rats. These data point to a calcium-dependent mode of action in the periphery (most probably in blood vessels) and the fact that ruthenium red and pentobarbitone behaved in a similar way as nifedipine are in harmony with this view (Kwolek *et al.*, 2005). In an attempt to further delineate this site of action, we studied the interaction of anandamide with a variety of antagonists in urethane-anaesthetized rats. The results suggest that α_1- and α_2-adrenoceptors, P2X/P2Y, neuropeptide Y_1, serotonin 5-HT_{2A}, vasopressin V_{1a}, endothelin and angiotensin AT_1 receptors are not involved in the pressor response to AEA (Kwolek *et al.*, 2005). There is some evidence to suggest that the vasoconstriction in the spleen vascular bed of urethane-anaesthetized rats, which is insensitive to CB_1 receptor and sympathetic blockade (Wagner *et al.*, 2001), has the same mechanism.

In the paper by Kwolek *et al.* (2005) and in a subsequent study (Malinowska *et al.*, 2010), the AEA-stimulated pressor action in urethane-anaesthetized, as opposed to pithed, rats was counteracted by antagonists of NMDA receptors, β_2-adrenoceptors and thromboxane A_2 (TP) receptors – MK-801, ICI 118551 and sulotroban respectively. These data suggest that central NMDA receptors, β_2-adrenoceptors and TP receptors may also play a role in the pressor effect of AEA. In order to further substantiate this hypothesis experiments were carried out in which AEA was administered i.c.v. Since i.c.v. AEA elicited two subsequent short-lived and small hypotensive effects only, the TRPV1 and CB_1 receptors (major

components of phase I and III) were blocked and under this condition indeed a marked pressor effect was obtained. The latter was counteracted by i.v. administration of an NMDA, β_2 or TP receptor antagonist. For the thromboxane A_2 system, we could show that AEA and MethAEA are devoid of an affinity at the TP receptor; they may rather lead to an increase in thromboxane A_2 formation (Malinowska *et al.*, 2010).

The mechanism of action, the interplay of the three receptors and the anatomical site(s) could not be proven by the study by Malinowska *et al.* (2010). Anandamide may act as a positive allosteric modulator at NMDA receptors (which in turn have a stimulatory effect on BP); evidence that AEA may act as a positive allosteric modulator is available from the literature (Hampson *et al.*, 1998; Mukhtarov *et al.*, 2005). The NMDA receptor may be the first step followed by TP and β_2 receptors and the paraventricular nucleus of the hypothalamus (PVN; an important brain area for BP control) may be the primary site of action.

So far, the vasopressor response to AEA and its metabolically stable analogue has been considered only. However, Δ^9-THC and Δ^8-THC can also elicit a vasopressor response in urethane-anaesthetized rats (Adams *et al.*, 1976; Lake *et al.*, 1997a) in a manner insensitive to rimonabant (Δ^9-THC; Lake *et al.*, 1997a). In the study by Adams *et al.* (1976), an increase in the perfusion pressure of the hindquarters vascular bed was obtained after i.a. injection of Δ^9-THC into the hind limb. This effect was counteracted by the α-adrenoceptor antagonist phentolamine and by pretreatment of the animals with reserpine, suggesting that the vasopressor effect in this case is based on the displacement of noradrenaline from the vascular sympathetic nerve endings by a carrier-mediated release (reverse direction of transport by the neuronal noradrenaline transporter, NAT; Figure 3).

Phase III

The prolonged hypotension induced by AEA (phase III) has been examined most extensively among the three phases so far. Like phase II, it is probably stimulated by complex mechanisms involving different receptors.

The main mechanism responsible is the activation of CB_1 receptors since the hypotension was blocked by the CB_1 receptor antagonists rimonabant or AM251 (Varga *et al.*, 1995, 1996; Vidrio *et al.*, 1996; Lake *et al.*, 1997a; Malinowska *et al.*, 2001a; Kwolek *et al.*, 2005; Zakrzeska *et al.*, 2010) and was absent in $CB_1^{-/-}$ mice (Járai *et al.*, 1999; Ledent *et al.*, 1999). In addition, there is a positive correlation between the EC_{50} values of various cannabinoid receptor agonists in producing hypotensive and bradycardic responses and in their affinity constants for binding to CB_1 receptors: HU210 > CP55940 > WIN55212-2 > Δ^9-THC > AEA (Lake *et al.*, 1997a).

Almost from the beginning on, the involvement of presynaptically located CB_1 receptors in phase III was postulated (Figure 3). In a series on intact and barodenervated urethane-anaesthetized rats, Varga *et al.* (1996) could exclude a site within the CNS as the target area for AEA. Instead, these receptors are located in the sympathetic nervous system. This is also suggested by a broad body of experiments in pithed animals and *in vitro*. So CP55940 and WIN55212-2 failed to affect the isoprenaline- or noradrenaline-stimulated tachycardia and pressor response, respectively, excluding postsynaptic sites of action. On the other hand, they inhibited the electrically stimulated increase in BP, HR and/or plasma noradrenaline concentration in pithed rats (Malinowska *et al.*, 1997, 2001b; Niederhoffer *et al.*, 2003) and rabbits (Niederhoffer and Szabo, 1999, 2000; Szabo *et al.*, 2001) in a manner sensitive to rimonabant. Moreover, Δ^9-THC, AEA or WIN55212-2 decreased the release of [^{3}H]-noradrenaline from isolated rat (Ishac *et al.*, 1996), guinea pig (Kurz *et al.*, 2008) and human (Molderings *et al.*, 1999; Table 2) atria and from vessels of the guinea-pig (Schultheiß *et al.*, 2005). All the above effects were diminished by rimonabant.

With respect to the location of the presynaptic inhibitory CB_1 receptors in the sympathetic nervous system *in vivo*, several possibilities have to be considered (Figure 3). These receptors are probably located on the postganglionic sympathetic nerve endings since the inhibitory effect mediated via these receptors was detected not only after electrical stimulation of the pre-ganglionic sympathetic nerve fibres in pithed rabbits or rats but also if the post-ganglionic fibres were stimulated electrically (Szabo *et al.*, 2001) or by injection of nicotine (Malinowska *et al.*, 2001b). However, an additional pre-ganglionic localization cannot been excluded. Moreover, presynaptic inhibitory CB_1 receptors located on the pre-ganglionic sympathetic nerve fibres projecting to the *adrenal medulla* may contribute since CP55940 and WIN55212-2 decreased plasma adrenaline release in pithed rabbits and in their isolated adrenal glands in a manner sensitive to rimonabant (Niederhoffer *et al.*, 2001). Presynaptic inhibitory CB_1 receptors have also been demonstrated on heart *vagal* fibres in pithed rabbits (Figure 3; Szabo *et al.*, 2001) but not in pithed rats (Malinowska *et al.*, 2001b). When present, these receptors may limit the extent of bradycardia accompanying phase III since they should functionally counteract the bradycardia elicited by CB_1 receptors located on sympathetic nerves and causing inhibition of noradrenaline and/or adrenaline.

In a study on urethane-anaesthetized pithed rats, we found that MethAEA inhibited the nicotine-stimulated tachycardia in a manner not sensitive to the CB_1 receptor antagonist AM251 (Baranowska *et al.*, 2008). The data of that study suggest that this inhibitory effect is related to a negative allosteric effect of MethAEA at α7-nicotinic acetylcholine receptors. Thus, a competitive antagonist at the latter receptors, methyllycaconitine, inhibited the nicotine-induced tachycardia to the same extent as MethAEA and the effects of both compounds were not additive. Moreover, it is known from studies in *Xenopus* oocytes that MethAEA (like anandamide but unlike Δ^9-THC, CP55940 and WIN55212-2) allosterically inhibits α7-nicotinic acetylcholine receptors (Oz *et al.*, 2003, 2004). Taken together, both CB_1 receptors and an allosteric binding site at α7-nicotinic acetylcholine receptors can be implicated in the presynaptic inhibition of the neurogenic tachycardia of the heart (Figure 3).

Postsynaptic myocardial CB_1 receptors (Figure 3) which cause negative inotropy in rats are suggested to be also involved in phase III under certain circumstances (e.g. hypertension or septic shock; for review, see Bátkai and Pacher, 2009). Thus, it has been found in experiments in which a sophisticated pressure-volume analysis system was applied to anaesthetized rodents that the hypotensive effect of AEA could be attributed to a CB_1 receptor-mediated decrease in cardiac contractility rather than to a decrease in vascular

Table 2

Occurrence of components of the endocannabinoid system in human cardiovascular tissues[1]

Component	Parameter	Function	Tissue	References
AEA	Compound	n.d.	V. saphena vascular endothelium	Bilfinger *et al.* (1998)
2-AG	Compound	n.d.	Aortic smooth muscle and umbilical vein endothelial cells	Sugiura *et al.* (1998)
CB_1 receptor	mRNA, protein	n.d.	Left ventricular myocardium	Weis *et al.* (2010)
	Function	Inhibiton of NAdr release	Atrial appendages	Molderings *et al.* (1999)
	Protein	Decrease in contractility	Atrium	Bonz *et al.* (2003)
	Function	AEA- and 2-AG-stimulated NO release	Right atrium	Bilfinger *et al.* (1998); Stefano *et al.* (2000)
	Protein	AEA-stimulated NO release	Internal thoracic artery	Bilfinger *et al.* (1998)
	Function	2-AG stimulated NO release	V. saphena vascular endothelium	Bilfinger *et al.* (1998); Stefano *et al.* (2000)
	mRNA	n.d.	Aortic smooth muscle and umbilical vein endothelial cells	Sugiura *et al.* (1998)
	mRNA	n.d.	Hepatic artery	Liu *et al.* (2000)
	Protein	n.d.	Coronary artery smooth muscle cells	Rajesh *et al.* (2007)
	mRNA, protein	n.d.	Cerebromicrovascular endothelial cells	Golech *et al.* (2004)
CB_2 receptor	mRNA, protein	n.d.	Left ventricular myocardium	Weis *et al.* (2010)
	Protein	n.d.	Coronary artery smooth muscle cells	Rajesh *et al.* (2007)
	mRNA, protein	n.d.	Cerebromicrovascular endothelial cells	Golech *et al.* (2004)
CB_x receptor	Function	Relaxation	Pulmonary artery	Kozłowska *et al.* (2007, 2008)
TRPV1 receptor	mRNA, protein	n.d.	Cerebromicrovascular endothelial cells	Golech *et al.* (2004)

[1]Note that this table also comprises mechanisms of the endocannabinoid system not considered in the rest of this review that is focussing on *haemodynamic* aspects of cannabinoids. E.g., Rajesh *et al.* (2007) showed that CB_2 receptors regulate vascular smooth muscle proliferation and migration. For some of the entries, a functional effect *in vitro* and/or *in vivo* has so far not been shown.
n.d., not determined; NAdr, noradrenaline.

resistance (Bátkai *et al.*, 2004; Pacher *et al.*, 2005; Bátkai and Pacher, 2009). CB_1 receptors leading to a decrease in contractile performance have also been detected in human atrial tissue (Bonz *et al.*, 2003; Table 2).

Importantly, AEA caused a stronger hypotension in anaesthetized spontaneously hypertensive rats (SHR) compared with normotensive animals (Lake *et al.*, 1997a; Bátkai *et al.*, 2004). In addition, the CB_1 receptor antagonists rimonabant and AM251 increased BP and cardiac contractility not in normotensive anaesthetized rats but in three models of hypertensive anaesthetized animals (SHR, Dahl salt-sensitive rats, rats with angiotensin II-induced hypertension; Bátkai *et al.*, 2004). Similarly, the FAAH inhibitor AM3506 normalized the elevated BP and cardiac contractility of SHR without affecting those parameters in normotensive rats (Godlewski *et al.*, 2010).

The prolonged hypotension might also be elicited by the activation of cannabinoid CB_1 and/or vanilloid TRPV1 receptors in the spinal cord since intrathecal (i.t.) administration (at the level of T_{12}-L_1) of WIN55212-2, AEA, MethAEA, NADA and capsaicin to urethane-anaesthetized rats produced hypotensive effects (García *et al.*, 2003; 2009). The above responses were abolished/reduced by nicotinic ganglion blockade with hexamethonium and by the CB_1 receptor antagonist rimonabant (against WIN55212-2) and the TRPV1 receptor antagonist capsazepine (against capsaicin). Authors concluded that the fall in BP elicited by AEA (i.t.) is related to the release of calcitonin-gene-related-peptide (CGRP) followed by the release of GABA in the spinal cord since it was mimicked by CGRP and prevented by $GABA_A$ as well as $GABA_B$ receptor antagonists.

A direct vasodilator effect may also contribute to the prolonged fall in BP elicited by AEA (Zakrzeska *et al.*, 2010). In urethane-anaesthetized rats, the decrease in BP elicited by AEA and MethAEA was counteracted by the antagonists of CB_x receptors, cannabidiol and O-1918, suggesting that these receptors contribute to the effect of AEA (Figure 3). The effect of the two antagonists was retained in pithed rats, excluding an interaction in the central nervous system. The possibility that the effect of the two antagonists to some extent involves CB_1 receptors can be discarded since cannabidiol and O-1918 at the doses used were ineffective in a CB_1 receptor paradigm in pithed rats in the same study. Importantly, vasodilatory CB_x receptors have also been demonstrated in human pulmonary arteries (Kozłowska *et al.*, 2007, 2008; Table 2). The possibility that other vasodilator mechanisms activated by cannabinoids and identified *in vitro* (for review, see Randall *et al.*, 2004; Mendizábal and Adler-Graschinsky, 2007) may come into play has also to be considered (for human vessels, see Table 2). However, so far, in *vivo* data are not available.

A special comment to 2-AG is necessary here. In contrast to AEA, its cardiovascular effects have been less examined although its level in the brain and in the periphery is higher than that of AEA (e.g. Di Marzo *et al.*, 2005). In addition, the blood level of 2-AG increases more dramatically under various pathological states than that of AEA (e.g. Kase *et al.*, 2008; Weis *et al.*, 2010). Bolus i.v. injection of 2-AG in rats anaesthetized with urethane (Varga *et al.*, 1998) or in mice anaesthetized with pentobarbitone (Járai *et al.*, 2000) induced monophasic changes in cardiovascular parameters, that is, hypotension connected with moderate tachycardia (Table 1). They lasted for about 10 min. Their mechanisms have been examined in detail in mice only (Járai *et al.*, 2000). Thus, the hypotension induced by 2-AG was insensitive to the CB_1 receptor antagonist rimonabant and was retained in $CB_1^{-/-}$ mice but was significantly diminished by the cyclooxygenase inhibitor indomethacin. On the other hand, the 2-AG-stimulated tachycardia was not modified by indomethacin but reduced by rimonabant (probably related to the rimonabant-elicited increase in basal HR), although it still occurred in $CB_1^{-/-}$ mice. Authors concluded that the hypotension is related to rapid degradation of 2-AG to a hypotensive arachidonic acid metabolite, whereas the mechanism of the tachycardia remains unclear (Járai *et al.*, 2000). Interestingly, a metabolically stable ether analogue of 2-AG decreased BP and HR via CB_1 receptors; that is, the changes were blocked by rimonabant and absent in $CB_1^{-/-}$ mice (Járai *et al.*, 2000). In rats, rimonabant diminished the 2-AG-induced hypotension without affecting the tachycardia (Varga *et al.*, 1998).

In summary, in anaesthetized rodents, the short-lived decrease in HR and BP obtained only with AEA, and its stable analogue MethAEA (phase I) is related to the activation of TRPV1 receptors (Bezold–Jarisch reflex). A short-lived increase in BP (phase II) was obtained with AEA, MethAEA and Δ^9-THC only. The mechanism is poorly understood so far, but the involvement of CB_1 receptors was excluded. There is good evidence to suggest that there are peripheral and central components. It is even unclear whether AEA (and MethAEA) on the one hand and Δ^9-THC on the other share the same mechanism(s). The prolonged hypotension (phase III) obtained with each of the cannabinoids AEA, MethAEA, Δ^9-THC, CP55940, HU210 and WIN55212-2 is related to the activation of peripheral CB_1 receptors including presynaptic CB_1 receptors on the sympathetic nerves innervating the resistance vessels and the heart. CB_1 receptors leading to a direct negative inotropic effect and other mechanisms may contribute.

Influence of cannabinoids on cardiovascular parameters in conscious animals

The reason why the cardiovascular effects in anaesthetized and conscious animals are described in separate sections is that there are very marked differences. The most prominent effect in anaesthetized rodents is a prolonged hypotension, whereas in conscious rodents, a pressor response prevails. How can we explain this discrepancy? The most obvious reason is the direct influence of anaesthetic agents on components of the endocannabinoid system. Thus, urethane, but not pentobarbitone, attenuates presynaptic CB_1 receptor function (Kurz *et al.*, 2009), which is important for phase III. On the other hand, pentobarbitone, but not urethane, inhibits the pressor effect of AEA (Kwolek *et al.*, 2005), which is important for phase II. In addition, anaesthesia *per se* affects the resting sympathetic tone, which is higher under the influence of urethane than in unstressed conscious rats (Carruba *et al.*, 1987). It has already been mentioned that phase III is related to the inhibition of the sympathetic tone. Moreover, the complex haemodynamic effects of AEA, that is two hypotensive responses separated by a hypertensive one, causes that a factor that reduces the fall in BP (e.g. the blockade of CB_1 receptors) may automatically enhance the preceding pressor effect and *vice versa*.

As shown in Table 1, in conscious rats AEA, MethAEA, Δ^9-THC, WIN55212-2 and HU210 elicited a brief pressor response connected with renal and mesenteric vasoconstriction and hindquarters vasodilatation (Lake *et al.*, 1997b; Gardiner *et al.*, 2001, 2002a,b, 2009; O'Sullivan *et al.*, 2007) (phase II). An initial bradycardia, hypotension and hindquarters vasoconstriction were also observed in response to AEA, especially its higher doses (phase I); however, unlike in anaesthetized rats, none of the five cannabinoids led to a prolonged hypotension (phase III). Mesenteric vasoconstriction and hindquarters vasodilatation of AEA were augmented by the inhibition of FAAH with URB597 but not by COX-2 inhibition with parecoxib (Gardiner *et al.*, 2009); these data suggest that AEA is degraded to a marked extent by FAAH under the experimental conditions chosen, but that formation of prostanoids from the degradation product arachidonic acid does not occur. Surprisingly, the TRPV1 receptor antagonist capsazepine failed to modify the cardiovascular effects of AEA (including phase I) (Gardiner *et al.*, 2009). The authors concluded that either there is no TRPV1 receptor involvement in the cardiovascular actions of AEA, or that the dose of capsazepine was inadequate under the conditions of their experiment.

Rimonabant slightly increased and prolonged the pressor effect of AEA (Lake *et al.*, 1997b), suggesting that this pressor effect is partially masked by peripheral CB_1 receptors leading to an inhibition of noradrenaline release. Another CB_1 receptor antagonist, AM251, failed to affect the increase in BP and all vasoconstrictor responses induced by i.v. AEA injection (Gardiner *et al.*, 2002a) but diminished all cardiovascular effects of Δ^9-THC (O'Sullivan *et al.*, 2007), WIN55212-2 and HU210 (Gardiner *et al.*, 2002b) and, interestingly, also those elicited by MethAEA (Gardiner *et al.*, 2009). The actions of WIN55212-2 were also abolished by the ganglion blocking agent pentolinium pointing to sympathoexcitation as the underlying mechanism (Gardiner *et al.*, 2001). The hindquarters vasodilatation induced by all cannabinoids under study involves β_2-adrenoceptors. It has been also demonstrated that none of the cardiovascular effects of AEA was modified by the serotonin 5-HT_3 receptor antagonist azasetron (Gardiner *et al.*, 2002a). In contrast to the i.v. injection of AEA, its local i.a. administration in the decerebrate rat model (in the absence of anaesthesia) elicited an increase in BP that involves predominantly CB_1 and partially TRPV1 receptors located on group IV primary afferent fibres (Williams *et al.*, 2008; Table 1).

Unlike in conscious normotensive animals, prolonged hypotension and bradycardia (phase III) were elicited in rats made acutely hypertensive by infusion of angiotensin II plus vasopressin by AEA and WIN55212-2 (Ho and Gardiner, 2009) and in SHR by AEA (Lake *et al.*, 1997b) and the new FAAH inhibitor AM3506 (Godlewski *et al.*, 2010). The depressor effects were connected with vasodilatation in the renal, mesenteric and hindquarters vascular beds (Ho and Gardiner, 2009). Rimonabant or AM251 blocked the above cardiovascular responses to AEA (only in SHR; Lake *et al.*, 1997b), WIN55212-2 (Lake *et al.*, 1997b; Ho and Gardiner, 2009) and AM3506 (Godlewski *et al.*, 2010) without affecting those elicited by AEA in acutely hypertensive rats (Ho and Gardiner, 2009). The bradycardic response to Δ^9-THC was enhanced by AM251 (O'Sullivan *et al.*, 2007), suggesting that CB_1 receptors may be coupled to a positive chronotropic activity, either centrally or directly. Taken together, the data suggest that in conscious rodents phase I involves TRPV1 receptors (although final proof is missing), phase II central CB_1 receptors (this mechanism is agonist-specific and is not valid for AEA) and phase III (occurring in animals with an elevated blood pressure only) peripheral CB_1 receptors. In addition, non-CB_1 receptor-mediated mechanisms may be involved in the cardiovascular effects of AEA both in normotensive and in acutely hypertensive rats since CB_1 receptor antagonists failed to block cardiovascular effects of this agonist (see above, Gardiner *et al.*, 2002a; Ho and Gardiner, 2009). Locations of the central CB_1 receptors leading to an increase in blood pressure have been examined in a series of studies in which cannabinoids have been administered topically to sites within the brain; these results will be discussed in the next few paragraphs.

Almost all experiments in which cannabinoids were applied centrally support the hypothesis that central mechanisms contribute to phase II (Figure 3, Table 1). In the study by Niederhoffer and Szabo (2000), cannabinoids have been administered into the *cisterna magna* (i.c.) of conscious rabbits; this procedure allows to examine their effects on cardiovascular centres in the medulla oblongata. I.c. injection of CP55940 and WIN55212-2 elicited a CB_1 receptor-dependent sympathoactivation manifested by increases in mean BP, plasma noradrenaline concentration and renal sympathetic nerve activity (RSNA) and by a vagally mediated bradycardia. Since the same pattern of changes has also been found in response to WIN55212-2 in urethane-anaesthetized rats (Pfitzer *et al.*, 2004; for details see Table 1), one can probably exclude the possibility that the sympathoexcitation is related to the state of consciousness rather than to a central site of cannabinoid actions. However, the above effects were accompanied by decreases in the respiratory rate and minute volume and all changes were sensitive to CB_1 receptor blockade. Thus, Pfitzer *et al.* (2004) concluded that the pressor effect of the cannabinoid may result from the activation of cardiovascular centres in the brain stem, but it can also be the response to the respiratory depression.

A key centre in the CNS responsible for the tonical pressor activity of the sympathetic nerve fibres is the rostral ventrolateral medulla (RVLM) (Figure 3). The brief pressor response to AEA i.v. was preceded by a transient rise in the activity of the RVLM and a brief rise in splanchnic sympathetic nerve discharge in urethane-anaesthetized rats (Varga *et al.*, 1996). In addition, the direct microinjection of WIN55212-2 and HU210 into the RVLM increased BP and renal sympathetic nerve activity in a CB_1 receptor-dependent manner; CB_1 receptor gene expression in the RVLM has also been shown (Padley *et al.*, 2003).

Sympathoexcitation can also result from the activation of the periaqueductal gray (PAG) (Figure 3), a mesencephalic region that has been proposed to play a role in specific cardiovascular changes associated with different emotional behaviours observed during the defense reaction. It has been demonstrated in a recent paper (Dean, 2011) that microinjection of AEA into the defense pathway of the dorsal PAG (dPAG) resulted in an increase in BP and RSNA in a manner sensitive to the CB_1 receptor antagonist AM281. In addition, AM281 injected into the dPAG attenuated the increase in BP and RSNA induced by electrical stimulation of the dPAG (Dean, 2011).

CB_1 receptors have also been found in the nucleus tractus solitarii (NTS; Brozoski *et al.*, 2009), that is a medullary area where various cardiac sensory afferents terminate including those of the baroreceptor, chemoreceptor and Bezold–Jarisch reflex (Figure 3). In anaesthetized rats (Niederhoffer *et al.*, 2003; Brozoski *et al.*, 2009) and dogs (Rademacher *et al.*, 2003) microinjection of AEA, AM404 (an inhibitor of endocannabinoid transport and probably also of FAAH) or WIN55212-2 into the NTS elicited no effects. However, AEA and AM404 injected into the NTS prolonged the reflex inhibition of the RSNA in a manner sensitive to rimonabant (Brozoski *et al.*, 2009). It is suggested that an acute increase in BP leads to the activation of presynaptic CB_1 receptors that alter the release of GABA and hence prolong the baroreflex-related sympathoinhibition (for detailed discussion, see Brozoski *et al.*, 2009).

At the end of this section, two cannabinoids that have so far not been mentioned in this review will be discussed (for their chemical structure, see Figure 1). Oleamide, which possesses affinity both for TRPV1 and CB_1 receptors, failed to affect BP and HR in conscious rats at 35 and 71 $\mu mol \cdot kg^{-1}$ administered i.p. although it elicited a series of centrally mediated effects, for example locomotor activity (Huitrón-Reséndiz *et al.*, 2001). By contrast, NADA i.v. elicited a triphasic BP response in conscious rats; the BP pattern closely resembles that obtained with AEA in anaesthetized rats, but the prolonged hypotension is accompanied by a tachycardia (Wang and Wang, 2007). The mechanisms involved in the three phases have been disclosed only partially. Interesting enough, on the basis of experiments with capsazepine TRPV1 receptors do not appear to be involved in phase I and II but play a role in phase III. On the other hand, the involvement of CB_1 receptors in the hypotension in phase III (and also in the BP responses in phase I and II) was excluded by experiments with rimonabant. The lack of contribution of CB_1 receptors to the hypotension in phase III is not so surprising since NADA activates CB_1 receptors in higher concentrations only. The TRPV1 receptors involved in phase III act via the release of CGRP, which in turn leads to vasodilatation. This conclusion is based on experiments in which a CGRP receptor antagonist was used and an increase in the plasma level of CGRP was determined. No information is available with respect to the mechanism underlying the tachycardia in phase III. Note that the TRPV1 receptors involved in phase III

here act via a local antidromic reflex, whereas those leading to phase I and II in anaesthetized animals involve the CNS.

In summary, when comparing the data obtained on anaesthetized and conscious animals, phase I does not differ markedly. Extreme differences, however, appear with respect to phases II and III. Phase II becomes more prominent for AEA, MethAEA and Δ^9-THC and is also obtained with other cannabinoids (HU210, WIN55212-2); this stimulatory phase may be (with the exception of AEA) related to activation of CB_1 receptors in the brain. The reason why these receptors do not come into play in anaesthetized rats is so far unclear. Phase III is no longer detectable in conscious rats but re-appears when BP is increased. There is good evidence that this phase is unmasked only when the sympathetic tone is high; anaesthesia is a situation under which a high sympathetic tone occurs.

Influence of cannabinoids on cardiovascular parameters in humans

Cannabinoids exhibit cardiovascular effects also in humans. In this context, one has to take into consideration that the cannabinoids are restricted to cannabis preparations, Δ^9-THC and nabilone used for recreational and/or therapeutic purposes and that smoking is the most frequent route of administration. Compounds like AEA, MethAEA, CP55940, HU210 and WIN55212-2 do not play a role. Many components of the endocannabinoid system have also been shown in human tissues but only in few cases their function under *in vitro* conditions has been demonstrated (Table 2).

One of the well-known acute effects of cannabis preparations including their major psychotropic constituent Δ^9-THC (whether delivered orally, i.v. or through smoking) is a rapid and substantial dose-dependent increase in HR. It may be accompanied by a modest increase in BP (particularly when supine) and an increase in cardiac output. Tachycardia was also noticed after oral administration of the synthetic cannabinoid nabilone (Cesamet®; Lile *et al.*, 2011) or by the oromucosal spray Sativex® (containing Δ^9-THC: cannabidiol ≈ 1:1; Karschner *et al.*, 2011). A review of almost 200 articles describing almost 400 different tests demonstrated that an increase in HR was the most consistent result, and almost all studies with the measurement of this parameter proved statistically significant. Thus, an increase in HR is even believed to be a useful cannabinoid biomarker (Zuurman *et al.*, 2009). Although there is much inter-individual variability, typical increases in HR associated with a single marijuana cigarette range from 20% to 100%, with the peak in HR occurring 10 to 30 min after the onset of smoking. Tolerance to the acute cardiovascular effects of marijuana smoking develops over several days to a few weeks, but it is rapidly lost when cannabinoid administration is stopped (Benowitz and Jones, 1981).

In a study on male cannabis users, the tachycardia was markedly attenuated by orally administered rimonabant (Huestis *et al.*, 2007). Additional studies suggest that the tachycardia may be related to an increased sympathetic activity connected with catecholamine release and decreased parasympathetic autonomic activity. For example, the peak HR rise after Δ^9-THC was attenuated by atropine and by propranolol and nearly abolished by atropine-propranolol pretreatment (Benowitz *et al.*, 1979). However, the immediate tachycardia was not accompanied by an increase in noradrenaline levels, which increased 30 min after marijuana exposure only and remained elevated for at least 2 h (Gash *et al.*, 1978). In addition, it has recently been demonstrated in 16 volunteers that Δ^9-THC increased HR independent of sympathetic activity since catecholamine levels were not changed (Dumont *et al.*, 2009). Under such conditions, the tachycardia might be related to the CB_1 receptor-mediated inhibition of acetylcholine release from the parasympathetic nerves innervating the heart (Figure 3). Pacher *et al.* (2005) suppose that this mechanism may play a role particularly in healthy young adults whose heart is under a dominant vagal tone. The typical tachycardia obtained with Δ^9-THC or cannabis preparations cannot be related to a Δ^9-THC-triggered increase in carrier-mediated noradrenaline release (studied in rats; Figure 3); in this case, one would rather expect an increase in BP associated with a baroreceptor reflex-related decrease in HR. Finally, it is remarkable that combined administration of Δ^9-THC plus cannabidiol had the same effect as administration of Δ^9-THC alone. Cannabidiol is known to counteract many Δ^9-THC-induced effects in general (Russo and Guy, 2006) and to increase the baroreflex-induced bradycardia by the activation of 5-HT_{1A} receptors in rats (Alves *et al.*, 2010) (Table 1).

A second cardiovascular effect associated with marijuana smoking is orthostatic hypotension. The hypotension was greater and longer lasting in hypertensive compared with normotensive volunteers (Crawford and Merritt, 1979). It may occur as a result of decreased vascular resistance (e.g. Jones, 2002; Sidney, 2002; Mathew *et al.*, 2003). The Δ^9-THC-induced decrease in BP (Gorelick *et al.*, 2006) could be blocked by the CB_1 receptor antagonist rimonabant, again indicating the involvement of these receptors. Chronic use of cannabis may elicit a long-lasting decrease in HR and BP. Interestingly, both major alterations obtained with Δ^9-THC in humans, namely an increase in HR and a decrease in BP, have also been detected in conscious rhesus monkeys (Fredericks *et al.*, 1981; Table 1). However, unlike in humans, both phenomena occurred in combination. A rapid tolerance developed to the tachycardia, whereas the hypotension did not change upon repeated administration of Δ^9-THC (Fredericks *et al.*, 1981).

Severe acute cardiovascular events may be associated with marijuana as described by some clinical reports. Thus, a decrease in the exercise time to angina, cardiac arrhythmias, ventricular tachycardia, palpitations or atrial fibrillation have been noticed (Caldicott *et al.*, 2005; Aryana and Williams, 2007). Moreover, it caused a 4.8-fold increase in the risk of myocardial infarction within 1 h after marijuana smoking (Mittleman *et al.*, 2001). Marijuana use was also associated with a threefold greater mortality after myocardial infarction (Mukamal *et al.*, 2008) and ischemic stroke (Singh *et al.*, 2011). However, the exact aetiopathology of these changes still remains to be established. The following mechanisms are proposed: cardiac ischaemia due to an increase in HR, postural hypotension, proarrhythmic effect of catecholamines, delay in seeking medical care for acute coronary events due to the analgesic properties of cannabinoids, impaired oxygen

supply to the heart secondary to increases in blood carboxyhaemoglobin levels and cellular stress elicited by oxidant gases produced by marijuana. Transient vasospasm is suggested as a cause for stroke (Caldicott *et al.*, 2005; Aryana and Williams, 2007). The well-known reduction in cerebral blood flow may contribute as well.

In summary, in humans the cannabinoids Δ^9-THC (alone or in combination with cannabidiol) or nabilone are used only. They frequently lead to a tachycardia sometimes associated with a modest increase in blood pressure. Rarely, they lead to decrease in blood pressure. There are two major differences with respect to animals. First, the two alterations do not occur in combination. Second, the stimulation of the cardiovascular system primarily involves an increase in heart rate rather than in blood pressure. There are, however, also remarkable similarities to the situation in conscious animals. Thus, like in conscious animals, 'phases II' and 'III' may be related to the activation of CB_1 receptors. 'Phase II' in humans is frequently related to an excitation of the sympathetic and depression of the parasympathetic nervous system. In some instances, presynaptic inhibitory CB_1 receptors on the parasympathetic nerve fibres supplying the heart (decelerator nerves) may be involved. 'Phase III' may be related to the activation of inhibitory presynaptic CB_1 receptors on the sympathetic nerve fibres innervating resistance vessels and heart. Like in animals, this phase is more marked under hypertension.

Acknowledgements

The authors would like to thank the Deutsche Forschungsgemeinschaft (DFG) and the Fundacja na rzecz Nauki Polskiej (FNP) (Copernicus Award jointly to BM and ES), the Medical University of Bialystok (F-13954F to BM) and the DFG (Forschergruppe 926, Schl 266/7-1 to ES) for the financial support they received for their research quoted in this review article.

Conflicts of interest

None.

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