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Review article

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ROLE OF ENDOCANNABINOIDS IN CARDIOVASCULAR SHOCK

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Endocannabinoids (*e.g.* anandamide, 2-arachidonoylglycerol or virodhamine) regulate the function of the cardiovascular system mainly in the following way: 1) by acting *via* CB_1 receptors, 2) by activation of CB_2 receptors, and 3) by modifying the function of vanilloid TRPV1, serotonin 5-HT_3 and α_7-subunit-containing nicotinic acetylcholine receptors. Endocannabinoids are implicated in the pathogenesis of hypertension and of hypotension associated with haemorrhagic, endotoxic, and cardiogenic shock, and with advanced liver cirrhosis. There is also evidence for their involvement in the control of atherosclerosis.

Key words: *endocannabinoids, cannabinoid receptors, anandamide, septic shock, cardiogenic shock, haemorrhagic shock, cardiovascular system*

We dedicate this paper to the memory of our colleague Dr Bogna Kamila Koneczny.

INTRODUCTION

Preparations of *Cannabis sativa* (hashish, marijuana) have for centuries been used for medical and recreational purposes. The major psychoactive component of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC), and synthetic cannabinoids (*e.g.*, WIN 55,212-2 and CP-55,940) act *via* two G protein-coupled receptors identified by molecular cloning: the neuronal CB_1 receptor, mediating most of the neurobehavioral effects of cannabinoids and found predominantly in the brain and in the peripheral nervous system, and the extraneuronal CB_2 receptor demonstrated mainly in immune and haematopoietic tissues. Soon after the discovery of these receptors, their endogenous ligands, named endocannabinoids

(*e.g.*, anandamide, 2-arachidonoylglycerol [2-AG] or virodhamine) with cannabimimetic activity were identified as metabolites of arachidonic acid (for review, see 1-7).

INFLUENCE OF CANNABINOIDS ON THE CARDIOVASCULAR FUNCTION UNDER PHYSIOLOGICAL CONDITIONS

Besides their neurobehavioural and immunological actions, cannabinoids exert important cardiovascular effects, encompassing modulation of autonomic outflow in the central and peripheral nervous system as well as direct effects on myocardium and vasculature, which have been described in detail in many reviews (e.g. see 1-7). As shown in *Table 1*, cardiovascular effects of cannabinoids can be mediated (in the broadest sense) *via* CB_1 and CB_2 receptors, the so-called endothelial cannabinoid receptor, ionotropic receptors such as vanilloid TRPV1, serotonin 5-HT_3 and α7-nicotinic receptors and still other mechanisms.

Influence of cannabinoids on the human cardiovascular system

Several effects of smoked marijuana on the cardiovascular system have been described (see *e.g.* 8, 9). Although there is much interindividual variability, typical increases in heart rate associated with a single marijuana cigarette range from 20% to 100%, with the peak in heart rate occurring 10 to 30 minutes after beginning to smoke. In addition, most subjects experience an increase in blood pressure, particularly when supine. Orthostatic hypotension may occur acutely as a result of decreased vascular resistance. Smoking marijuana decreases exercise test duration in maximal exercise tests and increases the heart rate at submaximal levels of exercise. Chronic use of cannabis in man elicits a long-lasting decrease in heart rate and blood pressure. Tolerance develops to the acute effects of marijuana smoking and Δ^9-THC over several days to a few weeks.

The mRNA for cannabinoid CB_1 receptors was detected in human aorta (10) and hepatic artery (11). In addition, the expression of cannabinoid CB_1, CB_2 and vanilloid TRPV1 receptor mRNA was demonstrated on human cerebromicrovascular endothelial cells (12). However, so far, the influence of endocannabinoids on human vessels was examined in few studies only. Thus, the endocannabinoid anandamide relaxed isolated human pulmonary artery rings (13) but not myometrial arteries isolated from pregnant women (14). Its application on the human skin (unlike its intravasal administration) increased microcirculatory flow (15). We showed that another endocannabinoid, virodhamine, and abnormal cannabidiol relaxed the human pulmonary artery *via* endothelial cannabinoid receptors (13, 16). The presence of anandamide, anandamide amidohydrolase (FAAH; the enzyme responsible for degradation of anandamide) and mRNA for cannabinoid CB_1 receptors have been also detected in human heart (17, 18). In

Table 1. Receptors involved in the cardiovascular effects of cannabinoids.

Type of receptor	Agonists	Antagonists	Effect	Mechanism	Literature (examples)
Presynaptic CB_1	WIN 55,212-2 CP 55,940 AEA MAEA	Rimonabant AM 251 AM 281	1. Prolonged hypotension and bradycardia. 2. Decrease in cardiac contractility.	Inhibition of NA release from the sympathetic nerve fibers innervating resistance vessels and heart.	19[1], 42[2], 43[2] 56[2], 57[2]
Postsynaptic CB_1			1. Vasorelaxation and decrease in blood pressure. 2. Decrease in cardiac contractility. 3. Cardioprotection.	Release of NO.	18[1],58[2], 59[2] 23[2], 26[2],47[2] 41[2]
CB_2		SR 144528 AM 630	Cardioprotection.	Activation of p38 MAPK. Release of NO. Reduction of cardiac arrhythmia.	2[2] 29[2] 30[2]
Endothelial cannabinoid	AEA abn-cbd Virodhamine	O-1918 Cannabidiol Rimonabant (high concen-tration)	Vascular relaxation.	Relaxation dependent on endo-thelium (e.g. release of endothe-lium-derived hyperpolarizing factor).	60[2], 13[1], 16[1]
Vanilloid TRPV1	AEA MAEA	Capsazepine Ruthenium red	1. Stimulation of an immediate transient decrease in heart rate accompanied by a fall in blood pressure (Bezold-Jarisch reflex). 2. Vasodilatation. 3. Increase in blood pressure.	1. Activation of vanilloid $TRPV_1$ receptors located on sensory vagal nerves in the heart. 2. Release of CGRP from sensory nerve endings. 3. Unknown.	49[2] 50[2] 61[2],62[2]
α7-Nicotinic acetylcholine	MAEA	*Rimonabant without ant-agonistic effect*	Inhibition of the nicotine-induced tachycardia.	Inhibition of α7 subunit-containing nicotinic acetylcholine receptors on sympathetic nerve fibers.	63[2]
Serotonin 5-HT_3	WIN 55,212-2 CP 55,940	*Rimonabant without ant-agonistic effect*	Inhibition of the phenylbiguanide-induced reflex bradycardia.	Inhibition of serotonin 5-HT_3 receptors on sensory vagal nerves in the heart.	64[2]
Other recep-tors/mecha-nisms	AEA AEA		1. Negative inotropic and/or coronary vasodilator responses 2. Increase in blood pressure	1. Non CB_1/CB_2 receptors 2. Central (probably medulla oblongata) and peripheral site of action (probably in blood vessels), exact mechanisms unknown	65[2] 20[2],62[2]

[1]Demonstrated in human tissue, [2]Demonstrated in animal models *in vitro* or *in vivo*.
abn-cbd – abnormal cannabidiol; AEA – anandamide; CGRP – calcitonin gene-related peptide; MAEA – methanandamide; MAPK – mitogen activated protein kinase

addition, functional presynaptic inhibitory cannabinoid CB_1 receptors have been identified in human heart (19). Moreover, anandamide was found to decrease cardiac contractility not only in rats (20) but also in humans (18).

INFLUENCE OF CANNABINOIDS ON THE CARDIOVASCULAR FUNCTION UNDER PATHOPHYSIOLOGICAL CONDITIONS

Endocannabinoids play a role in the cardiovascular system not only under physiological but also under pathophysiological conditions. Pathophysiological conditions comprise several forms of shock in the broadest sense (see next paragraph and *Table 2*) but also hypertension. With respect to the latter condition, endocannabinoids limit the pathological increase in blood pressure and in cardiac contractile performance. In support of this, anandamide caused a larger and longer lasting hypotension in anaesthetized spontaneously hypertensive rats (SHR) compared with anaesthetized normotensive rats (21, 22). The hypotensive action of anandamide was observed in conscious SHR but not in conscious normotensive rats (21, 23). The cannabinoid CB_1 receptor antagonist rimonabant increased blood pressure and cardiac contractility in three different models of anaesthetized hypertensive animals, i.e. SHR, Dahl salt-sensitive rats and rats with angiotensin II-induced hypertension (22). The expression of CB_1 receptors was increased in the myocardium and aortic endothelium of SHR compared with normotensive controls (22). Prevention of the degradation or uptake of anandamide by treatment with the FAAH inhibitor URB597 or the transport inhibitor OMDM2 reduced blood pressure, cardiac contractility and vascular resistance in SHR to the level observed in normotensive controls in a CB_1 receptor antagonist-sensitive manner (22). The simultaneous analysis of changes in blood pressure and cardiac performance showed that a decrease in cardiac contractility rather than a reduction in peripheral resistance was primarily responsible for the antihypertensive effect of anandamide (22).

Endocannabinoids exert a cardioprotective effect mainly *via* cannabinoid CB_2 receptors. Thus, in the rat heart anandamide and 2-AG have been shown to limit infarct size (24-26). Moreover, the cannabinoid CB_2 antagonist SR 144528 abolished the protective effect of endotoxin/lipopolysaccharide (LPS; 27) and heat stress (28) against myocardial ischaemia-reperfusion injury. Δ^9-THC protected neonatal cardiomyocytes against hypoxia *via* cannabinoid CB_2 receptor activation by induction of NO production (29). However, the delayed (24 h) preconditioning through transdermal nitroglycerin application increased the rat heart tissue content of 2-AG (but not anandamide), which reduced the left ventricular infarct size *via* CB_1 receptors (26). It has been also shown that the cannabinoid receptor agonist HU-210 exerted an antiarrhythmic effect during ischemia-reperfusion in rats *in vivo* (30).

Table 2. Participation of endocannabinoids in the pathology of shock and of hypotension associated with advanced liver cirrhosis and acute pancreatitis.

Pathophysiological state	Level of endocannabinoids		Modification of endocannabinergic mechanisms		Other effects	Literature
	AEA	2-AG	Antagonist[d]	Effect		
Cardiogenic shock[a]	↑ monocytes ↑ platelets	↑ monocytes ↑ platelets	Rimonabant	↑ BP, ↑ HR, ↓ endothelium-dependent relaxation, ↓ survival rate		41
Septic shock[a,b]	↑ macrophages	↑ platelets	Rimonabant AM281	↑ BP, ↑ cardiac contractility, ↑ survival rate ↑ BP, ↑ blood flow in aorta, carotid and renal arteries, ↑ arterial oxygenation, ↓ lactate overproduction, ↓ body temperature, ↑ survival rate		38, 47 36, 66
Septic shock[c]	↑ (4x) blood	↑ (3x) blood			↓ blood FAAH mRNA	67, 68
Haemorrhagic shock[a]	↑ macrophages		Rimonabant	↑ BP, ↑ HR, ↓ survival rate		35
Liver cirrhosis[a]	↑ (3x) heart ↑ (3.5x) liver	= heart ↑ (6x) liver	AM251	↑ BP, ↑ cardiac contractility	= myocardial CB_1R	69
	↑ (2-3x) monocytes		Rimonabant	↑ BP, ↓ mesenteric blood flow and portal pressure		40
			AM251	↑ reactivity to KCl and ↓ hyperresponse to acetylcholine in mesenteric arteries	↑ CB_1R in mesen-teric arteries	70
Liver cirrhosis[c]	↑ (10x) monocytes ↑ blood				↑ CB_1R in hepatic artery endothelium	40,71
Acute pancreatitis[a]	↑ blood		AM251	↑ BP, ↑ survival rate	= CB_1R in renal and pulmonary arteries	72

[a]rat; [b]mouse; [c]human being; acute administration[d]

= , ↓ and ↑ – no alteration, decrease and increase; AEA – anandamide; 2-AG – 2-arachidonoylglycerol; FAAH – fatty acid amide hydrolase; BP – blood pressure; HR – heart rate; CB_1R – cannabinoid CB_1 receptor

Endocannabinoids also exert a neuroprotective effect, mainly *via* activation of cannabinoid CB_1 receptors; *e.g.* in cannabinoid CB_1 receptor knockout mice, an increased severity of stroke was noticed (for review, see 3, 31). Furthermore, it has been demonstrated that low-dose oral cannabinoid therapy *via* CB_2 receptors reduces progression of atherosclerosis in mice (32).

Finally, the question arose whether the greater incidence of hypertension and coronary artery disease in men and in postmenopausal women could, at least partially, be related to changes in the function of the endocannabinoid system. Accordingly, estrogen as a putative protective factor was found to stimulate anandamide release from human blood endothelial cells (33) and anandamide-elicited vasorelaxation was greater in mesenteric arteries isolated from female than from male rats, suggesting a crucial dependence on the presence of estrogens (34).

Role of endocannabinoids in shock

Endogenous cannabinoids are implicated in the hypotension associated with different kinds of animal shock and with the fall in blood pressure in animal models of liver cirrhosis and acute pancreatitis (*Table 2*). Endocannabinoid-mediated cardiovascular effects even appear to influence survival since in the presence of rimonabant (given before shock) an increase in mortality, despite the increase in blood pressure, was reported in haemorrhagic and cardiogenic shock (*Table 2*). The cannabinoid agonists Δ^9-THC and HU-210 decreased the mortality in rats with haemorrhagic shock (35). In septic shock and in the hypotension connected with severe acute pancreatitis rimonabant or the CB_1 antagonists AM 281 or AM 251 decreased mortality. With respect to the sepsis model, the favourable effect of AM 281 on mortality was higher in non-diabetic than in diabetic rats (36). Importantly, hemoperfusion with polymyxin B-immobilized fiber, which results in anandamide (but not 2-AG) absorption, improved 28-day survival and organ failure in patients with sepsis (37). Unexpectedly, Δ^9-THC (like rimonabant) decreased mortality in rats with septic shock (38).

In several studies, the contribution of the endocannbinoids to the development of shock and hypotension was examined in detail. Thus, circulating platelets and macrophages from rats with haemorrhagic, septic and cardiogenic shock and from cirrhotic animals had elevated levels of anandamide or 2-AG (*Table 2*). A higher level of anandamide (but not 2-AG) was also determined in macrophages treated *in vitro* with LPS (38, 39). In addition, injection of macrophages or platelets isolated from animals with cardiogenic, septic or haemorrhagic shock into normal rats lowered blood pressure. A higher anandamide level and a more pronounced hypotensive response in recipient rats was noticed when macrophages were taken from mice deficient in FAAH and stimulated *in vitro* with LPS when compared to LPS-treated macrophages isolated from wild-type littermates (39). Importantly, the participation of endocannabinoids in shock has been also determined in humans since in the serum of patients with endotoxic

shock levels of anandamide and 2-AG were higher and the concentration of FAAH mRNA was lower than in normal serum (*Table 2*). Plasma levels of anandamide were also enhanced in patients with cirrhosis (*Table 2*). In addition, monocytes isolated from cirrhotic patients (but not healthy volunteers) and given intravenously to recipient rats also caused a long-lasting hypotension (40).

It has been postulated that CB_1 receptors are involved in the hypotension induced by endocannabinoids released during shock. Thus, the CB_1 receptor antagonist rimonabant completely prevented or even reversed hypotension in all animal models of circulatory shocks mentioned above. Another argument for the role of CB_1 receptors is the observation that the effect of rimonabant in haemorrhagic shock was diminished by the cannabinoid agonist WIN 55 212-2 (35). Moreover, an overexpression of cannabinoid CB_1 receptors was found in vascular endothelial cells from human cirrhotic livers and in mesenteric arteries isolated from rats with biliary cirrhosis (*Table 2*).

The hypotension induced by LPS or by hemorrhage was reversed in a dose-dependent manner by the intravenous injection of rimonabant but not by its administration into the *cisterna magna* or the IVth cerebral ventricle (35, 38) suggesting that one or more peripheral mechanisms are involved in the above effects. The significance of the preexisting sympathetic tone on the hypotensive response to endotoxic shock was excluded in experiments on anaesthetized rats pretreated with phentolamine (to eliminate α-adrenoceptor vasoconstrictor tone) and then continuously infused with vasopressin to restore basal blood pressure to control values; thus, the hypotensive response to LPS-exposed macrophages was comparable in phentolamine-pretreated and in control animals (38).

Rimonabant, probably *via* postsynaptic CB_1 receptors, also almost completely prevented the drop in blood pressure in the rats receiving injections of macrophages or platelets, isolated from rats with septic or haemorrhagic shock, or from cirrhotic patients (35, 38, 40). Other changes related to septic shock (i.e. fall in cardiac contractility and in blood flow in aorta, carotid and renal arteries, deterioration of arterial oxygenation, lactate overproduction or increase in body temperature) were also sensitive to rimonabant or to the CB_1 receptor antagonist AM 281 (for details, see *Table 2*). CB_1 receptor antagonists also counteracted the decrease in cardiac contractility and the increase in mesenteric blood flow and portal pressure in cirrhotic rats as well as the hyporeactivity to potassium chloride and the hyperresponse to acetylcholine in vessels prepared from such animals (*Table 2*). By contrast, rimonabant has been shown to further impair the acetylcholine-induced relaxation of the rat aortic rings, previously attenuated by myocardial infarction (41).

We decided to check whether presynaptic inhibitory cannabinoid CB_1 receptors located on sympathetic nerve terminals innervating blood vessels and heart (*Table 1*) play a role during shock as well. For this purpose we used the model of pithed and vagotomized rats. This model has been previously used by us for the identification of inhibitory presynaptic CB_1 receptors on sympathetic

nerve fibres innervating resistance vessels (42) and heart (43). It offers the opportunity to study drug effects on the peripheral cardiovascular system without interference with reflex loops implicating the central nervous system. Electrical stimulation of the preganglionic sympathetic nerve fibres produced increases in blood pressure (BP) or heart rate (HR) by about 30 mmHg and 50 beats/min, respectively (for stimulation parameters and experimental protocol, see *Fig. 1* in (44) and legends to *Fig. 1* and *Fig. 2* of the present paper). We applied two models of shock, namely septic shock (induced by injection of LPS) and haemorrhagic shock (elicited by slow withdrawal of blood). Because of the lack of reflex responses in pithed as compared to anesthetized rats (35, 38), the visible sign of shock (i.e., the development of profound hypotension) was obtained already by relatively low doses of LPS or by the withdrawal of relatively low volumes of blood in the pithed rat model.

Injection of LPS produced a dose-dependent inhibition of the neurogenic (*i.e.* electrically stimulated) increases in BP (*Fig. 1A*, (44)) and HR (*Fig. 2A*). The inhibitory effect for the highest doses of LPS (BP - 4 mg/kg and HR - 1.5 mg/kg) was about 40-50%. Similarly, haemorrhagic shock caused inhibition of the neurogenic vasopressor response by about 40% (*Fig. 1B*). We can exclude the possibility that the inhibitory effects on the neurogenic vasopressor response and tachycardia were due to changes in basal cardiovascular parameters developing in shock. Thus, the typical increase in HR induced by cardiovascular shock, which has been also noticed in anaesthetized rats undergoing septic shock (38), was either not present or only very slight in pithed rats during septic or haemorrhagic shock, respectively. Moreover, the profound hypotension stimulated by shock was compensated by constant infusion of vasopressin (LPS-induced shock) or prostaglandin $F_{2\alpha}$ (haemorrhagic shock). Control rats received infusion of saline solution instead.

In order to answer the question whether the observed inhibition is related to a presynaptic site of action we performed additional experiments, in which increases in BP or HR were induced by injection of agonists of α-adrenoceptors (noradrenaline 1-3 nmol/kg or phenylephrine 10 nmol/kg) or β-adrenoceptors (isoprenaline 0.05-0.15 nmol/kg). The respective increases in BP or HR were about 30 mmHg (noradrenaline, phenylephrine) and 50 beats/min (isoprenaline), respectively, i.e. comparable to those obtained under electrical stimulation. Quite unexpectedly, although septic and haemorrhagic shock uniformly inhibit the neurogenic tachycardia and/or vasopressor response, we obtained three different effects of shock on the increases in BP and HR stimulated by exogenous ligands. Firstly, LPS (4 mg/kg) was without influence on the noradrenaline-stimulated increase in BP ((44), *Fig. 1A*), suggesting that septic shock inhibits the neuorogenic vasopressor response *via* a presynaptic mechanism. Secondly, LPS strongly and dose-dependently amplified the chronotropic response to the non-selective β-adrenoceptor agonist isoprenaline (*Fig. 2A*). Additional experiments with selective agonists and antagonists of β-adrenoceptors led us to the conclusion that the

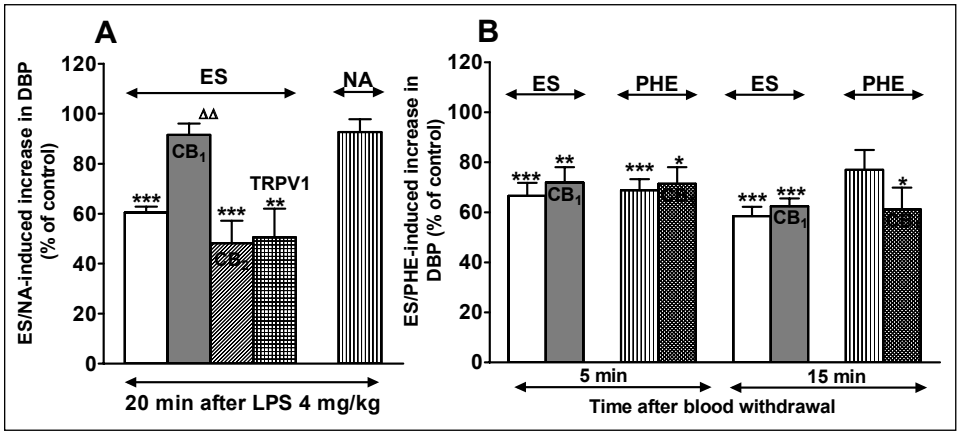

Fig. 1. Influence of lipopolysaccharide (LPS) (A) and blood withdrawal (B) on the increase in diastolic blood pressure (DBP) induced electrically (ES) or by intravenous (*i.v.*) injection of noradrenaline (NA) or phenylephrine (PHE) and interaction with the cannabinoid CB_1 receptor antagonist rimonabant (CB_1), the cannabinoid CB_2 receptor antagonist SR 144528 (CB_2) or the vanilloid TRPV1 receptor antagonist capsazepine (TRPV1) in pithed and vagotomized rats pretreated with pancuronium (0.8 μmol/kg). Septic shock was induced by LPS slowly injected *i.v.* over a time period of 1 min (A) whereas haemorrhagic shock was induced by withdrawal of 1.5 ml/kg blood over a time period of 2.5-3 min (B). Stimuli (ES: 1 Hz, 1 ms, 50 V [7-10 pulses] or NA 1-3 nmol/kg *i.v.* or PHE 10 nmol/kg *i.v.*) were administered 5 min before LPS or blood withdrawal (S_1) and were repeated 10, 20 and 30 min after induction of septic (S_2, S_3, S_4; only S_3 is shown here) and 5 and 15 min after induction of haemorrhagic shock (S_2, S_3). Rimonabant (0.1 μmol/kg) or SR 144528 (3 μmol/kg) were administered *i.v.* 10 min before S_1; capsazepine (1 μmol/kg) was administered 2 min before S_1 and 2 min before S_3. Both for animals exposed and not exposed to the antagonist, the ratios S_2/S_1 and S_3/S_1 for the increase in DBP obtained in rats with septic or haemorrhagic shock were expressed as percentages of the corresponding ratios obtained in control animals (vehicle for LPS; no blood withdrawal). Mean±SEM of 3-11 rats. *P<0.05, **P<0.01, ***P<0.001 compared to the corresponding control. $^{\triangle\triangle}$P<0.01 compared to the group not receiving rimonabant.

recruitment of functionally active heart β_2-adrenoceptors is responsible for the enhancement of the positive chronotropic response to β-adrenoceptor agonists in the initial phase of endotoxic shock in pithed rats (45). Thirdly, haemorrhagic shock inhibited the phenyleprine-elicited increase in blood pressure (to about the same extent as it inhibited the neurogenic vasopressor response; *Fig. 1B*).

Our next question concerned the possible involvement of endocannabinoids in the above effects. For this purpose, we used the CB_1 receptor antagonist rimonabant applied at the low dose of 100 nmol/kg known to block presynaptic CB_1 receptors (*e.g.* 43). The inhibitory effects of septic shock on the neurogenic pressor response and tachycardia were sensitive to rimonabant but were not modified by antagonists of CB_2 receptors (SR 144528), TRPV1 receptors (capsazepine) and/or histamine H_3 receptors (clobenpropit) (*Figs 1, 2* and (44)). Rimonabant (but not SR 144528) also

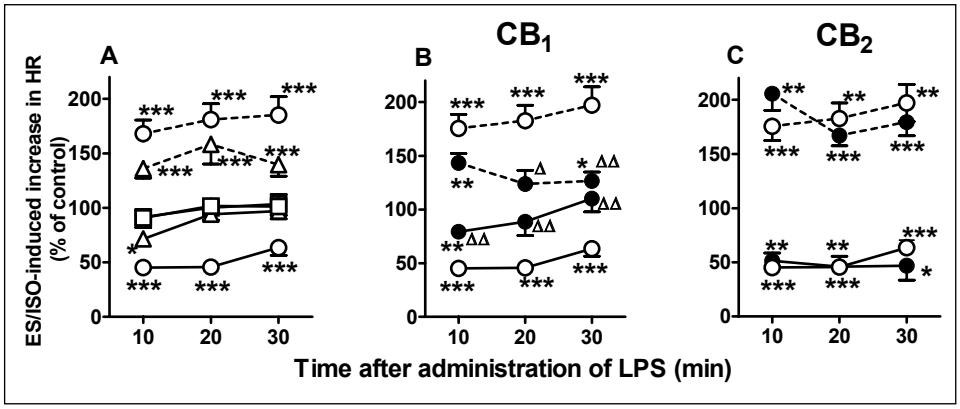

Fig. 2. Influence of lipopolysaccharide (LPS) on the increase in heart rate (HR) induced electrically (ES; *solid lines*) or by intravenous (*i.v.*) injection of isoprenaline (ISO; *broken lines*) and interaction with the cannabinoid CB_1 receptor antagonist rimonabant (CB_1; B) and the cannabinoid CB_2 receptor antagonist SR 144528 (CB_2; C) in pithed and vagotomized rats pretreated with pancuronium (0.8 μmol/kg; *i.v.*). Four stimuli (S_1-S_4) were applied (ES: 1 Hz, 1 ms, 50 V, 7 pulses; ISO: 0.05-0.15 nmol/kg). S_1 was administered 5 min before and S_2, S_3 and S_4 10, 20 and 30 min after injection of LPS, respectively. A single dose of LPS 0.4 (*squares*), 1.0 (*triangles*) and 1.5 mg/kg (*circles*) or its vehicle was injected (over a time period of 1 min) 5 min after S_1. Rimonabant (0.1 μmol/kg) or SR 144528 (3 μmol/kg) was administered *i.v.* 10 min before S_1. Both for animals exposed (*filled symbols*) and not exposed to the antagonist (*open symbols*), the ratios S_2/S_1, S_3/S_1 and S_4/S_1 for the increase in HR obtained in the presence of LPS were expressed as percentages of the corresponding ratios obtained in the animals treated with the vehicle for LPS (control). Mean±SEM of 3-11 rats. *P<0.05, **P<0.01, ***P<0.001 compared to the corresponding control. $^{\triangle}$P<0.05, $^{\triangle\triangle}$P<0.01 compared to the group not receiving rimonabant.

reduced the amplificatory influence of septic shock on the isoprenaline-stimulated positive chronotropic response (*Fig. 2B, C*). In contrast to septic shock, rimonabant failed to modify the inhibitory influence of haemorrhagic shock on the increases in blood pressure stimulated electrically and by injection of phenylephrine (*Fig. 1B*). None of the antagonists under study modified the electrically or agonist-induced increase in BP or HR by itself ((44), unpublished results).

Table 3 summarizes that presynaptic inhibitory CB_1 receptors that are located on pre- and/or postganglionic nerve fibres innervating resistance vessels and heart that serve as additional targets for endocannnabinoids released in the initial phase of septic, but not haemorrhagic, shock. Moreover, postsynaptic CB_1 receptors might be involved in the amplificatory influence of septic shock on the increase in HR directly stimulated *via* postsynaptic β-adrenoceptors by isoprenaline. The detailed mechanism underlying this interaction remains to be established. By contrast, isolated cirrhotic ventricular papillary muscles in hearts exhibited lower responsiveness to isoprenaline, which was completely restored by the CB_1 receptor antagonist AM 251 (46).

Table 3. Influence of septic and haemorrhagic shock on the neurogenic tachycardia and vasopressor responses in pithed rats and possible involvement of endocannabinoids in these effects.

Shock	Cardiovascular parameters	Effect	Mechanism	Literature
septic	Heart rate	*Post*synaptic increase	CB_1 receptor-mediated, CB_2 receptor excluded	Unpublished
		*Pre*synaptic decrease	CB_1 receptor-mediated, CB_2 receptor excluded	
	Diastolic blood pressure	No *post*synaptic effect		44
		*Pre*synaptic decrease	CB_1 receptor-mediated, CB_2, TRPV1 and H_3 receptors excluded	
hemorrhagic		*Post*synaptic decrease	Mechanism unknown, CB_1 receptor excluded	Unpublished
		*Pre*synaptic decrease	Probably result of the simultaneous postsynaptic decrease	

There is increasing evidence that, besides pre- and postsynaptic CB_1 receptors, also receptors similar although not identical to CB_1 receptors are involved in the shock-induced hypotension. Thus, quite unexpectedly LPS-induced hypotension in rats was reversed by rimonabant 3 mg/kg but not by the same dose of the CB_1 receptor antagonist AM 251. Moreover, the profound decrease in blood pressure elicited by LPS in anaesthetized mice deficient in CB_1 ($CB_1^{-/-}$) or both CB_1 and CB_2 ($CB_1^{-/-}$/$CB_2^{-/-}$) receptors was counteracted by rimonabant (47). The same group also observed in rats that rimonabant (but not another CB_1 receptor antagonist AM 251) strongly reversed the LPS-induced hypotension and the decrease in cardiac contractility but only slightly delayed the decrease in aortic blood flow and the increase in peripheral resistance and had no effect on the tachycardia elicited by LPS in rats (47). Thus, the authors concluded that rimonabant inhibits the acute hemodynamic effects of LPS by interacting with an unknown cardiac receptor distinct from CB_1 or CB_2 receptors. The presence of an additional non-CB_1-non-CB_2 cardiac cannabinoid receptor, mediating the anandamide-induced negative inotropic effect and coronary vasodilatation, has also been postulated on the basis of *in vitro* experiments (20). In LPS-stimulated hypotension the involvment of endothelial cannabinoid receptors is also likely since their antagonist O-1918 inhibited this effect (48).

Finally, as shown in *Table 1* anandamide is also an agonist of

vanilloid TRPV1 receptors located on sensory vagal nerves in the heart and on perivascular sensory nerves. Their activation causes reflex bradycardia and/or hypotension (49) and vasodilation through the release of calcitonin gene-related peptide (CGRP) (50), respectively. A series of studies suggests that TRPV1 receptors, in addition to CB_1 and cannabinoid-like receptors, are involved in cardiovascular effects induced by shock. Thus, the relaxant effect of anandamide in rat mesenteric arteries occurring in the early phase of endotoxic shock was connected with an overexpression of TRPV1 receptors, increased density of CGRP-positive nerves and enhancement of the anandamide-stimulated release of CGRP (51). Moreover, mesenteric arteries isolated from cirrhotic rats displayed overexpression of TRPV1 and CB_1 receptors. Moreover, the vasodilator response of these vessels to anandamide was reduced by antagonists of TRPV1 and CB_1 receptors (52). Recent studies indicate that TRPV1 receptors appear to play a protective role against endotoxin-induced hypotension and mortality. Thus, the LPS-induced fall in BP was higher in TRPV1 knockout than in wild-type conscious mice (53). Moreover, pretreatment of rats with the TRPV1 receptor antagonist capsazepine enhanced the LPS-elicited hypotension, reduced the endotoxemia-related increase in plasma noradrenaline and adrenaline levels and decreased the survival rate (54). During septic shock, an enhanced level of substance P was also observed. Moreover, two antagonists selective for substance P NK_1 receptors, RP-67580 and L-733,060, produced a similar pattern of changes

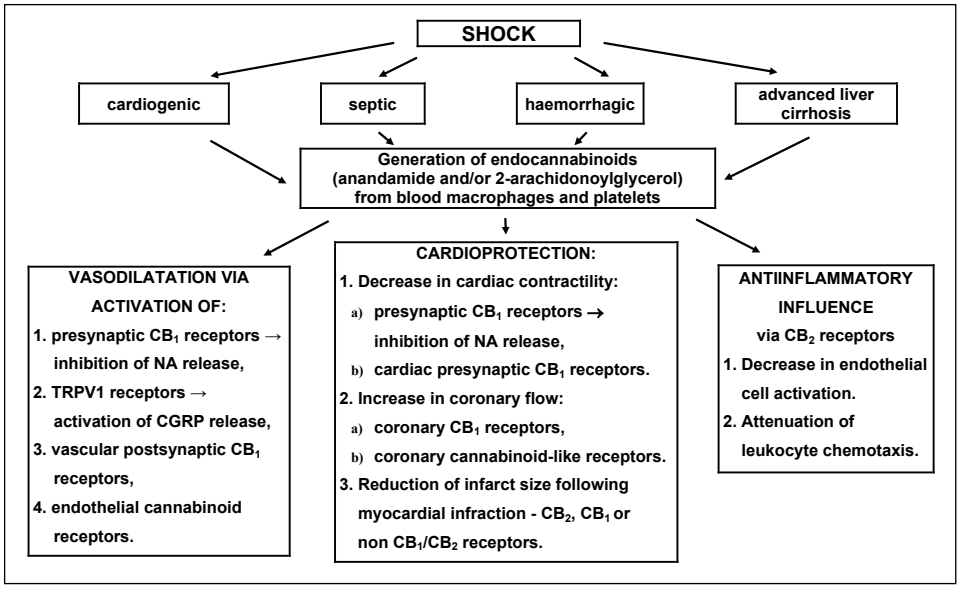

Fig. 3. Mechanisms involved in the cardiovascular effects of endocannabinoids during shock. The vasodilated state occurring during advanced liver cirrhosis has also been entered into this figure although this condition is not a shock *sensu stricto*.

in LPS-induced shock like capsazepine. These data suggest that an activation of TRPV1 receptors, mainly expressed is sensory nerves, probably leads to the release of substance P which activates NK_1 receptors and stimulates the sympathetic axis *via* different central and peripheral mechanisms (54).

Taken together, as shown in *Fig. 3* cardiogenic, septic and haemorrhagic shock or liver cirrhosis lead to the release of endocannabinoids (mainly anandamide and/or 2-AG) from macrophages and/or platelets. They cause vasodilatation and hypotension on the one hand, but exert a cardioprotective action on the other. In addition, they are known as anti-inflammatory substances acting mainly *via* CB_2 receptors (for review, see *e.g.* 5, 55). Endocannabinoids were found to modify the rate of survival. Thus, modulation of the endocannabinergic system may have a therapeutic implication in hypotension connected with various forms of shock.

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