

## EXPERIMENTAL STUDIES

# Endogenous Cannabinoids Mediate Hypotension After Experimental Myocardial Infarction

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<b>OBJECTIVES</b>	We sought to determine whether endocannabinoids influence hemodynamic variables in an experimental models of acute myocardial infarction (MI).
<b>BACKGROUND</b>	Hypotension and cardiogenic shock are common complications in acute MI. Cannabinoids are strong vasodilators, and endocannabinoids are involved in hypotension in hemorrhagic and septic shock.
<b>METHODS</b>	The early effect of left coronary artery ligation on hemodynamic variables was measured in rats pretreated with the selective cannabinoid <sub>1</sub> receptor (CB <sub>1</sub> ) antagonist SR141716A (herein referred to as SR, 6.45 μmol/kg body weight intravenously) or vehicle. Endocannabinoids produced in monocytes and platelets were quantified by liquid chromatography/mass spectrometry (LC/MS), and their effects on blood pressure and vascular reactivity were determined.
<b>RESULTS</b>	After MI, mean arterial pressure (MAP) dropped from 126 ± 2 mm Hg to 76 ± 3 mm Hg in control rats, whereas the decline in blood pressure was smaller (from 121 ± 3 mm Hg to 108 ± 7 mm Hg, <i>p</i> < 0.01) in rats pretreated with SR. SR increased the tachycardia that follows MI (change [Δ] in heart rate [HR] = +107 ± 21 beats/min vs. +49 ± 9 beats/min in control rats, <i>p</i> < 0.05). The MI sizes were the same in control rats and SR-treated rats. Circulating monocytes and platelets isolated 30 min after MI only decreased MAP when injected into untreated rats (ΔMAP = -20 ± 5 mm Hg), but not in SR-pretreated rats. The endocannabinoids anandamide and 2-arachidonyl glycerol were detected in monocytes and platelets isolated after MI, but not in cells from sham rats. Survival rates at 2 h after MI were 70% for control rats and 36% for SR-treated rats ( <i>p</i> < 0.05). Endothelium-dependent arterial relaxation was attenuated after MI (maximal relaxation: 44 ± 3% [ <i>p</i> < 0.01] vs. 70 ± 3% in control rats) and further depressed by SR treatment (24 ± 5%, <i>p</i> < 0.01 vs. MI placebo).
<b>CONCLUSIONS</b>	Cannabinoids generated in monocytes and platelets contribute to hypotension in acute MI. Cannabinoid <sub>1</sub> receptor blockade restores MAP but increases 2-h mortality, possibly by impairing endothelial function. (J Am Coll Cardiol 2001;38:2048-54) © 2001 by the American College of Cardiology

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In the first few hours after myocardial infarction (MI), cardiogenic shock characterized by inadequate cardiac output, profound hypotension and systemic hypoperfusion is a common clinical problem, as first described by Killip and Kimball in 1967 (1). Although the in-hospital mortality rate of patients with MI without heart failure was only 6%, the mortality rate of those who developed cardiogenic shock reached 81% (1). More recent data continue to show a grave prognosis for cardiogenic shock, with in-hospital mortality rates ranging from 43% to 73%. Even immediate coronary revascularization had no effect on 30-day survival and only a modest effect on six-month survival (2).

Beyond hemodynamic monitoring in specialized cardiac care units, use of catecholamines and careful control of fluid

balance, the therapeutic palette includes mechanical support by an intra-aortic balloon pump or left ventricular assist devices as bridge therapy to heart transplantation. However, a specific pharmacologic treatment to improve the poor prognosis and to reverse the hypotension after MI has yet to be introduced. Based on work using rodent models of MI, a number of mechanisms have been suggested to contribute to the development of congestive heart failure within weeks to a few months after MI. Surprisingly, very little is known about the possible mechanisms that account for the acute hypotensive phase after MI.

Cannabinoids are best known for their psychoactive properties, but they also influence cardiovascular variables (for review, see [3]). The recently discovered endocannabinoids anandamide (4) and 2-arachidonyl glycerol (2-AG) (5) act as natural ligands at specific cannabinoid<sub>1</sub> (CB<sub>1</sub>) (6) or CB<sub>2</sub> (7) receptors. They mimic most of the biologic effects of plant-derived and synthetic cannabinoids, including cardiovascular actions.

We recently reported that in rat models of hemorrhagic (8) and endotoxic shock (9), hypotension is prevented by

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#### Abbreviations and Acronyms

2-AG	= 2-arachidonyl glycerol
CB <sub>1</sub>	= cannabinoid <sub>1</sub> receptor
HR	= heart rate
IV	= intravenous
LC/MS	= liquid chromatography/mass spectrometry
MAP	= mean arterial pressure
MI	= myocardial infarction
SNP	= sodium nitroprusside

pretreatment with the selective CB<sub>1</sub> antagonist SR141716A (herein referred to as SR). In addition, circulating monocytes or platelets from rats in septic shock elicit CB<sub>1</sub> receptor-mediated hypotension when injected into normal rats, and these cells were found to generate anandamide and 2-AG (9). Intravenous (IV) injection of either anandamide (10) or 2-AG (9) has been shown to elicit hypotension that can be prevented by SR (11).

Therefore, in the present study, we investigated: 1) whether endocannabinoids and their specific receptors may mediate hypotension in cardiogenic shock; 2) whether early endothelial dysfunction contributes to that mechanism; 3) which cells may serve as a source of endocannabinoids; and 4) whether blockade of cannabinoid receptors restores blood pressure and improves early mortality.

## METHODS

**Surgical procedures.** All procedures were approved by the Institutional Animal Care and Use Committee and were in accordance with the "Position of the American Heart Association on Research Animal Use," adopted by the Association in November 1984. Adult female Wistar rats (weighing 275 to 300 g) were anesthetized with urethane (0.7 g/kg IV plus 0.3 g/kg intraperitoneally) and heparinized (500 IU/kg IV). Drugs or isolated blood cell preparations were injected through a polyethylene cannula in a femoral vein. Heart rate (HR) and mean arterial pressure (MAP) were monitored through a cannula inserted into the femoral artery and connected to a pressure transducer and physiograph. Coronary artery ligation was performed as previously described (12). Rats received either SR or vehicle (1:1:8 of ethanol/emulphor/saline; 0.2 ml) 15 min before coronary ligation. The dose of SR used (6.45 μmol/kg = 3 mg/kg IV) produced near-maximal inhibition of the hypotensive effect of anandamide (10), which is known to be mediated by CB<sub>1</sub> receptors (13). For survival studies, two additional groups of rats underwent left coronary ligation after pretreatment with vehicle or SR. The number of surviving rats was established over a 2-h observation period. The MI size was measured by triphenyltetrazolium chloride staining. For all studies, only rats with infarct sizes >33% were used.

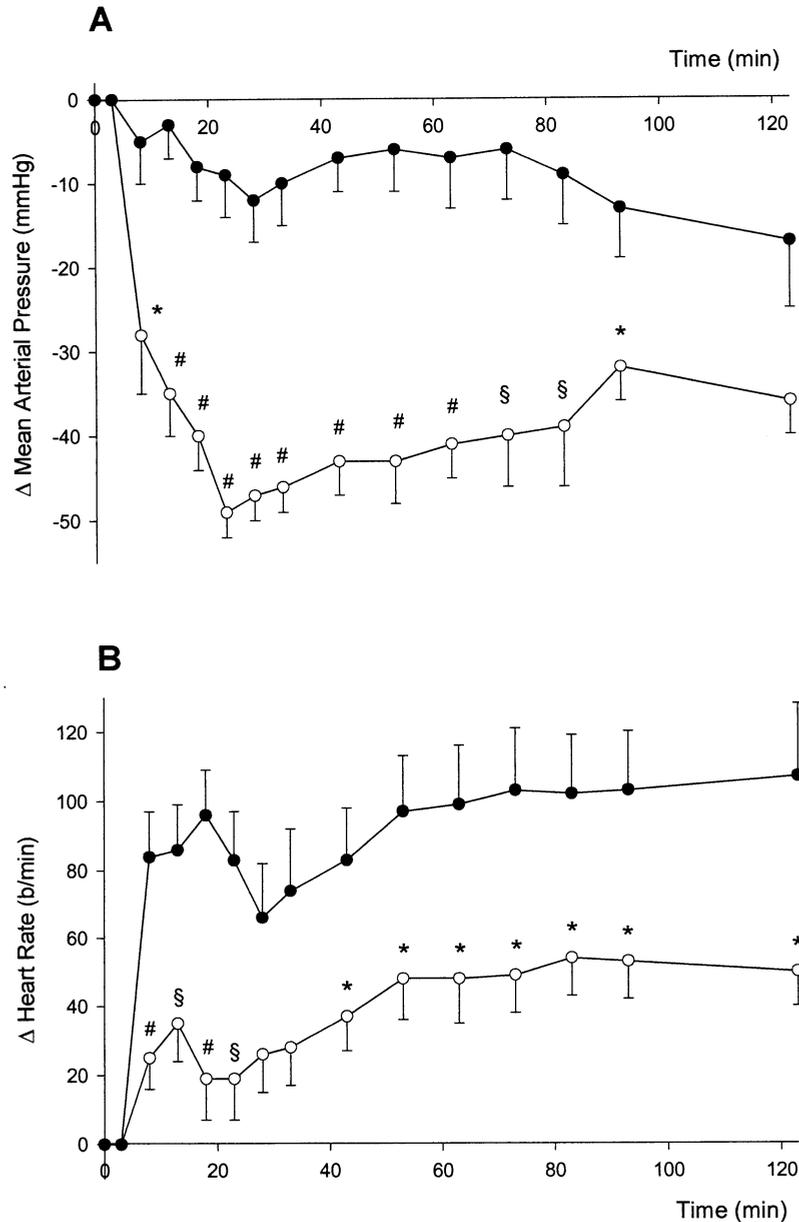
**Preparation of isolated blood cells.** In rats not used for hemodynamic measurements, 9 ml of blood was removed 30, 60 or 120 min after MI for isolation of platelets and

monocytes. Platelet-rich plasma was obtained by centrifugation at 100 × g for 15 min. The platelet-rich plasma was further centrifuged at 800 × g for 15 min, and the pellet was resuspended in 0.2 ml of phosphate-buffered saline. The mononuclear cell fraction was isolated from the remaining blood, as described in detail elsewhere (14). Cells were resuspended in 0.2 ml of phosphate-buffered saline and used for biochemical analysis or transfusion experiments. For the latter, both platelets and monocytes (~10<sup>6</sup>) were injected into recipient rats 15 min after SR (6.45 μmol/kg IV) or vehicle (control rats).

**Measurement of anandamide and 2-AG.** For liquid chromatography/mass spectrometry (LC/MS) analysis, monocytes or platelets were isolated from 25 to 30 ml of pooled normal or post-MI blood. The samples were spiked with 1 nmol of *d*-8-anandamide and 10 nmol *d*-8-2-AG as internal standards. Cells and medium were then extracted with chloroform/methanol (2:1 vol/vol). The organic phase was dried and resuspended in chloroform, to which ice-cold acetone was added to precipitate proteins. After removal of proteins by centrifugation, the organic phase was dried and resuspended in methanol for analysis by LC/MS. Cell extracts were fractionated by reverse-phase high-performance LC on an ODS column (Supelcosil, 5 μm, 4.6 mm × 15 cm), using a mobile phase of methanol/water/acetic acid (85:15:0.03 vol/vol/vol) at a flow rate of 1 ml/min. This was followed by in-line MS analysis on a Micromass Quattro II mass spectrometer equipped with an atmospheric pressure, chemical ionization source. Two selected ions were monitored simultaneously: selected on monitoring 387.2 for *d*-8-2-AG ions as the internal standard, selected on monitoring 379.2 for 2-AG ions, selected on monitoring 356.2 for *d*-8-anandamide ions as the internal standard and selected on monitoring 348.2 for anandamide ions.

**Vascular reactivity studies.** The descending thoracic aorta was dissected after removal and cleaned. Three-millimeter rings were mounted in an organ bath (Foehr Medical Instruments, Seeheim, Germany) for isometric force measurement. The rings were equilibrated for 30 min under a rest tension of 2 g in oxygenated Krebs-Henseleit solution containing diclofenac (1 μmol/l), as detailed elsewhere (15). The rings were repeatedly contracted with potassium chloride (100 mmol/l) until reproducible responses were obtained. Thereafter, the rings were precontracted with phenylephrine (0.3 to 1.0 μmol/l) to 80% of maximal contraction, and the relaxant response to the cumulative application of acetylcholine and sodium nitroprusside (SNP) was assessed.

**Drugs and chemicals.** SR141716A (*N*-[piperidin-1-yl]-5-[4-chlorophenyl]-1-[2,4-dichlorophenyl]-4-methyl-1H-pyrazol-3-carboxamide hydrogen chloride) was a gift from Sanofi Recherche (Montpellier, France). Anandamide, 2-AG, *d*-8-anandamide and *d*-8-2-AG were provided by Deva Biotech (Hatboro, Pennsylvania). Acetylcholine, SNP, phenylephrine, triphenyltetrazolium chloride and Histopaque-1077 were from Sigma.



**Figure 1.** The effects of myocardial infarction on mean arterial pressure (A) and heart rate (B) in control rats (open circles; n = 16) or SR141716A-pretreated (solid circles, n = 12) rats. SR141716A (6.45  $\mu\text{mol/kg}$  IV) was given 15 min before left coronary artery ligation, performed at 0 min. The vertical bars represent SEM. Significant differences (\*p < 0.05; §p < 0.01; #p < 0.001) from corresponding values in the absence of SR141716A.

**Statistical analyses.** The time-dependent effects of MI and drug interventions on blood pressure, HR and vascular reactivity were analyzed by analysis of variance, followed by the Bonferroni post-hoc test. Differences between two groups (SR or vehicle) or samples (with or without MI) were analyzed by the Student unpaired *t* test. Survival data were analyzed by the Fisher exact test.

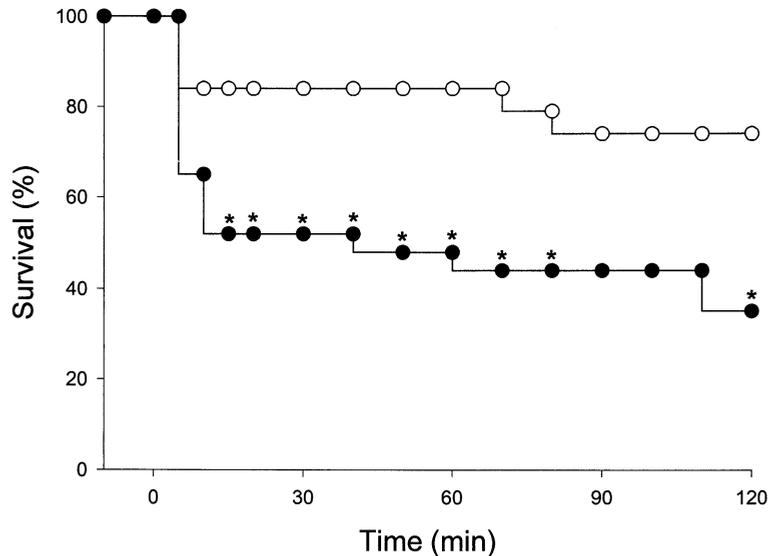
## RESULTS

There was no significant difference between the SR-pretreated group (n = 12) and the vehicle-treated control group (n = 16) regarding baseline MAP (121  $\pm$  3 mm Hg vs. 126  $\pm$  2 mm Hg), HR (380  $\pm$  10 beats/min vs. 392  $\pm$

11 beats/min), body weight (277  $\pm$  3 g vs. 284  $\pm$  9 g), heart weight (1.11  $\pm$  0.04 g vs. 1.2  $\pm$  0.04 g) and infarct size (39.9  $\pm$  3.6% vs. 38.3  $\pm$  3.7%).

**CB<sub>1</sub> receptor antagonist protects against post-MI hypotension.** Experimental MI caused immediate and long-lasting hypotension and moderate tachycardia (Fig. 1). SR almost completely prevented the drop in MAP and significantly enhanced the tachycardia. In agreement with earlier findings (8,9), SR alone did not significantly change HR or MAP.

**SR141716A enhances early mortality after MI.** Prompted by the restoration of MAP by SR pretreatment, we also tested its effect on mortality. The early survival rate was 70%



**Figure 2.** The effects of SR141716A (solid circles, n = 25) on early survival after myocardial infarction; MI was performed at 0 min. The rats were treated intravenously either with 6.45  $\mu\text{mol/kg}$  SR141716A or vehicle (open circles, n = 20) 15 min before experimental myocardial infarction. \*Significant difference ( $p < 0.05$ ) from corresponding value in vehicle-treated rats.

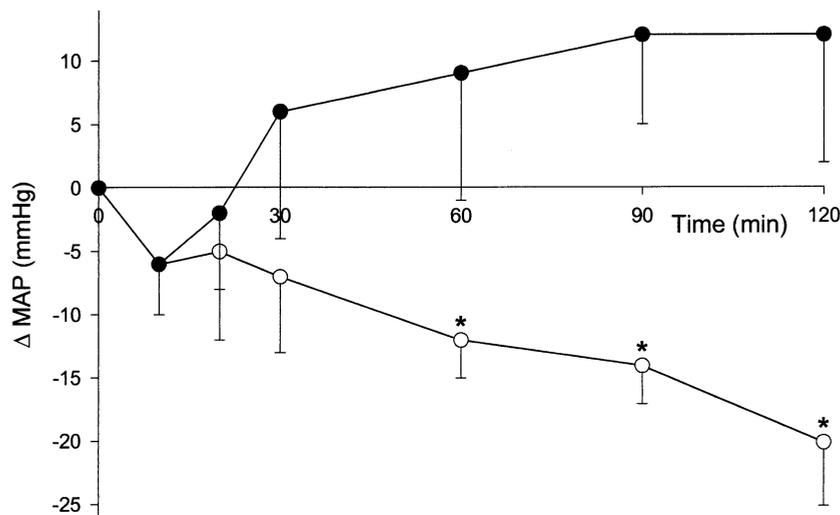
at 2 h after MI in untreated control rats, but only 36% in rats pretreated with the  $\text{CB}_1$  receptor antagonist ( $p < 0.05$ ) (Fig. 2).

**MI induces the generation of cannabinoids in circulating macrophages and platelets.** Isolated monocytes and platelets from rats with MI did not affect HR when injected into vehicle-treated control rats or rats pretreated with SR (data not shown). However, they caused gradually developing, prolonged hypotension in control rats, whereas in rats pretreated with SR, they caused enhanced MAP (Fig. 3). Monocytes and platelets from control rats without MI did not alter MAP or HR in recipients (data not shown).

Anandamide could be detected in samples from either

monocytes or platelets isolated after MI (Table 1), whereas in samples from untreated control rats or sham-operated rats, the anandamide content was below the limit of detectability of  $\sim 0.01$  pmol/ml (Table 1). Similar results were obtained for 2-AG, which could be identified in monocytes and platelets if isolated after MI, but not in cells from control rats or sham-operated rats (Table 1).

**Early endothelial dysfunction after MI is aggravated by SR141716A.** In post-MI rats, the vasodilatory response of aortic rings to acetylcholine was attenuated, as compared with that in sham-operated animals (Fig. 4A), whereas the relaxation with the endothelium-independent vasodilator SNP (Fig. 4B) was unchanged. Acetylcholine-induced re-



**Figure 3.** Changes in blood pressure of recipient rats in response to circulating monocytes and platelets isolated from rats with myocardial infarction. See Methods for details. Cells were injected into control (open circles, n = 5) and SR141716A-pretreated (6.45  $\mu\text{mol/kg}$  IV; solid circles, n = 4) rats at 0 min. Basal mean arterial pressure (MAP) and heart rate were  $116 \pm 5$  mm Hg and  $402 \pm 19$  beats/min before and  $114 \pm 7$  mm Hg and  $383 \pm 36$  beats/min after SR141716A, respectively \*Significant difference ( $p < 0.05$ ) from corresponding control value.

**Table 1.** Production of Anandamide and 2-Arachidonyl Glycerol in Platelets and Monocytes of Control, Sham or Coronary-Ligated Myocardial Infarction Rats

	Rats With MI			Sham-Operated Rats	Control Rats
	30 min	60 min	120 min		
Anandamide, platelets	0.48	< 0.01	0.08	< 0.01	< 0.01
Anandamide, monocytes	1.55	0.15	0.38	< 0.01	< 0.01
2-AG, platelets	3.6	13.1	7.1	< 0.01	< 0.01
2-AG, monocytes	8.7	1.1	9.8	< 0.01	0.01

Data are pmol for anandamide and 2-arachidonyl glycerol (2-AG) (cells in 1 ml of blood). Cells were isolated from blood pooled from three or four rats per group. Cells from rats with myocardial infarction (MI) were obtained at 30, 60, or 120 min after coronary artery ligation. In sham-operated and control rats, cells were obtained at 120 min only.

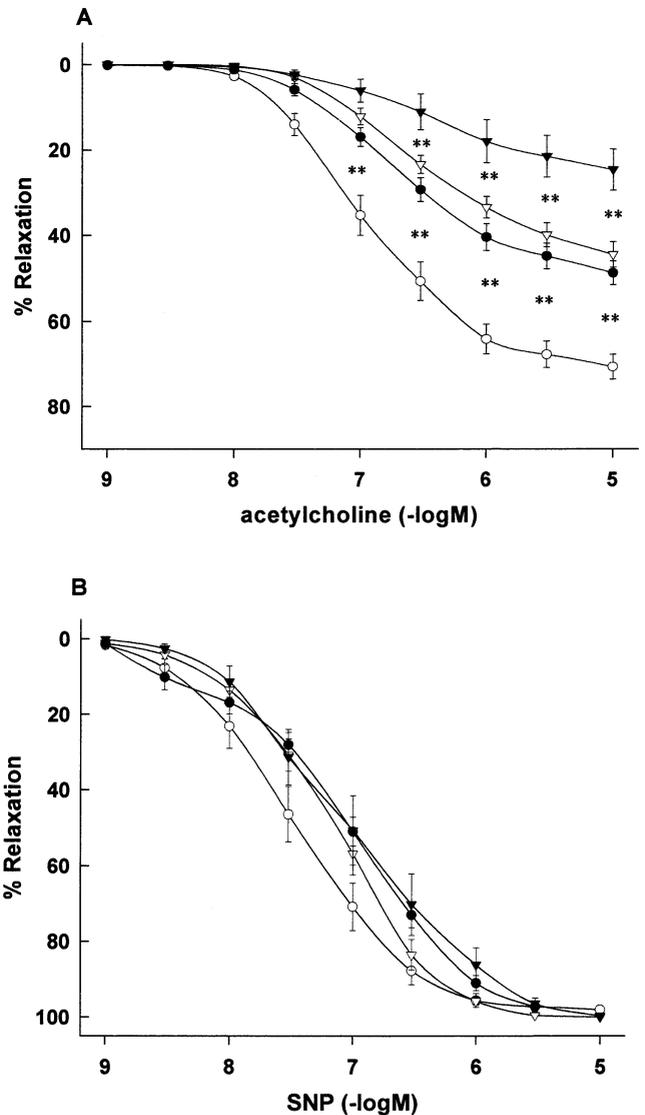
laxation was reduced after pretreatment with SR in sham-operated rats and further impaired in rats with MI, whereas SNP-induced responses were not altered (Fig. 4).

**DISCUSSION**

The findings indicate that activated vascular CB<sub>1</sub> receptors contribute to severe hypotension after experimental MI in rats. The endocannabinoids anandamide and 2-AG are generated by circulating monocytes and platelets during cardiogenic shock. The selective CB<sub>1</sub> receptor antagonist SR prevented post-MI hypotension, but aggravated early endothelial dysfunction and worsened mortality.

The high mortality associated with cardiogenic shock (1,2) and its resistance to current therapy justify the search for underlying mechanisms, and our working hypothesis was made plausible by growing evidence that endocannabinoids might be key players in the hemodynamic changes that accompany certain forms of shock (8,9). Cannabinoids are potent vasodilators and elicit hypotension and bradycardia (9,10). Inhibition of these effects by CB<sub>1</sub> receptor antagonists and their absence in CB<sub>1</sub> receptor knockout mice implicate CB<sub>1</sub> receptors (13). Activation of peripheral CB<sub>1</sub> receptors contributes to hypotension during hemorrhagic and septic shock (8,9). In both cases, the highly selective CB<sub>1</sub> receptor antagonist SR was able to restore blood pressure to control levels, and monocyte- and platelet-derived endocannabinoids have been identified as possible paracrine mediators (8,9). Here we extend this hypothesis to include cardiogenic shock.

**Similarities of endocannabinoid action in hemorrhagic, septic and cardiogenic shock.** First, CB<sub>1</sub> receptor antagonism prevents the hypotension that follows left coronary artery ligation (Fig. 1A). Unlike nitric oxide, cannabinoids are not tonically released, as indicated by the lack of change in blood pressure after SR treatment in control animals. Although the experimental MI model has been widely used to induce chronic congestive heart failure, little is known about the cellular/humoral factors that mediate the early decrease in blood pressure after MI. The observed ~50 mm Hg drop in MAP after MI is in agreement with the scarce published data (16,17). However, we noticed tachycardia rather than bradycardia (17), an



**Figure 4.** Relaxations induced by acetylcholine (A) and sodium nitropruside (SNP) (B) in phenylephrine-precontracted aortic rings from rats 2 h after myocardial infarction (solid and open downward triangles), as compared with sham-operated animals (solid and open circles). The rats were treated with either vehicle (open circles and open downward triangles) or SR141716A (6.45 μmol/kg intravenous 15 min before myocardial infarction (solid circles and solid downward triangles). The results are expressed as the mean value ± SEM from 8 to 12 separate experiments. \*\*p < 0.01 for vehicle used in rats with myocardial infarction vs. vehicle used in sham-operated rats vs. SR141716A used in rats with myocardial infarction.

effect further enhanced by previous CB<sub>1</sub> receptor blockade (Fig. 1B). This might be explained by antagonism of the known bradycardic effect of cannabinoids (10), which attenuate the tachycardic effect of sympathetic activation caused by the events associated with acute myocardial hypoxia.

Second, preventing the MI-induced blood pressure decrease by CB<sub>1</sub> receptor blockade had a detrimental effect on early survival (Fig. 2), similar to hemorrhagic shock (8). This is in agreement with the widely held view that restoration of blood pressure alone after MI offers no benefit, as

illustrated by the persistent high mortality rate in patients receiving high doses of catecholamines. In contrast, in hypovolemic and septic shock, pretreatment with the active ingredient of the marijuana plant,  $\Delta_9$ -tetrahydrocannabinol, improved survival (8,9). Shock-related hypotension triggers compensatory sympathetic vasoconstriction that can compromise the supply of oxygen to critical organs. Cannabinoids have been shown to dilate isolated coronary (18) and cerebral arteries (19) and recently have been shown to elicit strong cerebral and coronary vasodilation in vivo (20). It is plausible that endocannabinoid-induced vasodilation in cardiogenic shock helps to maintain adequate tissue perfusion in the face of decreased cardiac output and a compensatory increase in sympathetic vasoconstrictor tone, and its removal by SR pretreatment impairs survival.

As a further analogy with septic and hemorrhagic shock, the same cells are shown to generate endocannabinoids in cardiogenic shock. Monocytes and platelets from rats in cardiogenic shock cause a SR-dependent drop in MAP in normal recipient rats (Fig. 3). Furthermore, the same cells are shown to synthesize anandamide and 2-AG when isolated from rats in cardiogenic shock, but not from control rats (Table 1). Monocytes and platelets are known to display increased adherence to the vascular wall in septic (21) and hemorrhagic (22) shock. Likewise, cannabinoids released from adherent cells during cardiogenic shock may act as paracrine activators at vascular  $CB_1$  receptors. This mechanism would greatly enhance their local concentration at their vascular site of action, which could account for the substantial vasodilatory effect. The discovery of monocyte chemotactic and activating factor/monocyte chemoattractant protein-1 as an inflammatory mediator in patients with acute MI underscores the involvement of monocytes in the early stages of MI (23). As in septic shock, acute MI enhances through inflammatory responses and systemic hypoxia the release of monocyte-activating cytokines, such as tumor necrosis factor- $\alpha$  (24), and monocyte inhibitory cytokines, such as interleukin-10 (25). Also, cannabinoids themselves are able to alter the cytokine production of macrophages, with as-yet unknown consequences on blood pressure regulation (26).

We recently demonstrated that monocytes and platelets generate endocannabinoids in experimental as well as chronic human liver cirrhosis (27), which is often associated with endotoxemia. Patients in cardiogenic shock are exposed to bacterial endotoxin during bowel wall ischemia (28). Elevated plasma levels of endotoxin, either as a primary result of bacterial infection in septic shock or as a secondary consequence of ischemic, low-output conditions, may therefore be a common trigger for the production of endocannabinoids, which, in turn, may contribute to the associated hypotension.

**Do endocannabinoids ameliorate early endothelial dysfunction after MI?** Endothelial dysfunction contributing to elevated peripheral resistance is a common problem in congestive heart failure after MI (15). Here we show that in

rats with cardiogenic shock, endothelium-dependent but not direct vasorelaxation is selectively attenuated (Fig. 4). Similar observations have been made in hemorrhagic and endotoxemic shock (29). In septic shock, SR caused endothelium-dependent vasoconstriction in the mesenteric artery, possibly due to antagonism of the vasodilatory effect of a locally produced endogenous cannabinoid (30). Indeed, there is evidence that 2-AG is generated in vascular endothelial cells and contributes to the vasodilatory response to acetylcholine (31,32), which may explain the observed impairment of endothelial function after SR pretreatment in both sham rats and those with MI (Fig. 4A). In rats in cardiogenic shock, this further reduces the already impaired endothelial function, which may contribute to the increased mortality observed in rats with MI pretreated with SR.

**Clinical implications.** In the absence of rationally based treatment regimens, the outcome of patients in cardiogenic shock after MI remains abysmal. Therefore, the discovery of new mechanisms could offer novel approaches for developing therapeutic strategies. For example, nitric oxide synthase inhibitors are potent vasoconstrictors and are able to restore blood pressure in shock situations. Nevertheless, their effectiveness as standard therapeutic agents has been disappointing, although case reports and data obtained from small patient groups receiving multiple treatment regimens (33) may be encouraging.

The results of the present study show that endocannabinoids, a new class of neurohumoral vascular mediators, play a role in the hemodynamic variables of cardiogenic shock. Therefore, targeting their biosynthesis, specific receptors and biologic degradation systems may become a new strategy for the clinical management of cardiogenic shock.

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