Acute Myocardial Infarction Inhibits the Neurogenic Tachycardic and Vasopressor Response in Rats via Presynaptic Cannabinoid Type 1 Receptor

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ABSTRACT

The present study was carried out to examine whether acute experimental myocardial infarction affects the sympathetic transmission to vessels and the heart of pithed rats via a presynaptic mechanism and, if so, to check whether inhibitory presynaptic cannabinoid (CB) receptors and endocannabinoids are involved in this response. In pithed and vagotomized rats, electrical stimulation (0.75 Hz; 1 ms; 50 V: 5 or 15 pulses for increases in heart rate or blood pressure, respectively) of the preganglionic sympathetic nerve fibers or intravenous injection of isoprenaline (0.1 nmol/kg) or noradrenaline (1 nmol/kg) increased heart rate and blood pressure by approximately 50 beats/min and 40 mm Hg, respectively. Ligation of the left coronary artery reduced the electrically (as opposed to the chemically) induced tachycardic and pressor responses by approximately 30 to 40%. The inhibitory effect of myocardial infarction was prevented by the CB1 receptor antagonist rimonabant but not by the CB2 receptor antagonist N-[(1S)-endo-1,3,3-trimethyl-bicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyra-zole-3-carboxamide (SR144528) and the transient receptor potential vanilloid 1 receptor antagonist capsazepine. The inhibitory effect of myocardial infarction was slightly enhanced by the inhibitors of anandamide and 2-arachidonoylglycerol degradation, 3’-(aminocarbonyl)[1,1’-biphenyl]-3-yl-cyclohexylcarbamate (URB597) and 4-nitrophenyl-4-(dibenzo[d][1,3]dioxol-5-yl)(hydroxy)methyl)piperidine-1-carboxylate (JZL184), respectively. Rimonabant increased myocardial infarction-induced mortality. Our results demonstrate that during the early phase of myocardial infarction the activation of presynaptic CB1 receptors by endogenously formed cannabinoids contributes to the inhibition of the neurogenic tachycardic and vasopressor responses. Thus, the CB1 receptor-mediated inhibition of excessive noradrenaline release from the sympathetic nerve fibers innervating the heart and vessels might play a protective role in myocardial ischemia.
fecteds even seem to influence survival. Thus, mortality was increased in CB1 receptor knockout mice with acute heart failure induced by transverse aortic constriction (Liao et al., 2011) and in rats treated once with rimonabant before MI elicited by left coronary ligation (Wagner et al., 2001). On the other hand, there are case reports linking marijuana smoking (Caldicott et al., 2005; Tormey, 2012) or the use of the synthetic cannabinoid K2 (Mir et al., 2011) to the precipitation of angina and acute coronary syndromes. In a case-crossover study of 3882 patients, a 4.8-fold increase in the risk of MI was noticed in the first hour after smoking marijuana (Mittleman et al., 2001). Marijuana use was also associated with a 3-fold greater mortality after an infarction (Mukamal et al., 2008).

Exogenous and endogenously formed cannabinoids exert cardiovascular effects via CB1 receptors. Thus, it has been postulated that CB1 receptors are involved in the hypotension induced by endocannabinoids in an animal model of MI (Wagner et al., 2001), other forms of circulatory shock, including hemorrhagic shock (Wagner et al., 1997) and endotoxic shock (Bátkai et al., 2004; Godlewski et al., 2004), and the vasodilated state associated with advanced liver cirrhosis (Bátkai et al., 2001). We have shown that endocannabinoids activate vascular presynaptic CB1 receptors and inhibit the neurogenic vasopressor response in pithed rats, a mechanism contributing to the hypotension observed in the early phase of septic shock (Godlewski et al., 2004). In pithed rats, inhibitory presynaptic CB1 receptors are present on the sympathetic nerve endings innervating resistance vessels (Malinowska et al., 1997; Niederhoffer et al., 2003; Godlewski et al., 2004) and heart (Malinowska et al., 2001; Niederhoffer et al., 2003). Their activation leads to 1) the inhibition of noradrenaline (NA) release from the sympathetic nerve endings, 2) a diminished activation of postsynaptic α- and β-adrenoceptors on resistance vessels and heart, and 3) the reduction of neurogenic vasopressor and tachycardic responses, respectively. In addition to CB1, CB2 (Lépicier et al., 2006; Wang et al., 2012) and TRPV1 receptors (Lupiński et al., 2011) are involved in the cardiovascular effects of cannabinoids.

In acute MI, sympathetic activation accompanied by excessive noradrenaline release is a prominent cause of arrhythmias and cardiac dysfunction. Therefore, reduction of noradrenaline release from cardiac sympathetic nerves is an important protective mechanism (Tripodiadis et al., 2009). In the model of acute heart failure induced by transverse aortic constriction a deterioration associated with higher catecholamine levels was detected in CB1 receptor knockout mice (Liao et al., 2011), suggesting an influence of heart failure on presynaptic CB1 receptors. It is noteworthy that a decrease in presynaptic function was observed in patients with ischemic congestive heart failure (Caldwell et al., 2008). Moreover, the mechanism for coronary vasospasm after marijuana smoking may be related to an increase in sympathetic discharge (Gash et al., 1978).

Thus, the first aim of the present study was to examine whether acute experimental myocardial infarction in pithed rats inhibits neurogenic vasopressor and tachycardic responses. Because there were positive results, we next checked whether the observed effects could be overcome by selective antagonists of the CB1, CB2, and TRPV1 receptors. Because the latter experiments revealed that CB1 receptors are involved, we finally studied whether the inhibitory effect of MI on the neurogenic vasopressor and tachycardic responses can be further increased by blockade of the enzymes degrading the endocannabinoids AEA and 2-AG.

**Materials and Methods**

All surgical procedures and experimental protocols were approved by the local Animal Ethics Committee in Białystok (Poland). Male Wistar normotensive rats (Experimental Medical Centre, Białystok, Poland, weighing 220–350 g) with free access to food pellets and water were used in the experiments.

**General Procedure.** Rats were anesthetized by intraperitoneal injection of pentobarbitone sodium (300 μmol/kg). Then they were injected with atropine (2 μmol/kg i.p.). After cannulation of the trachea the animals were pithed by inserting a stainless-steel rod through the orbit and foramen magnum into the vertebral canal. The pithing rod had a diameter of 1.5 mm and a length of 190 mm and was enameled except for a uncovered segment situated at vertebrae C7–T1 or T9–L1 in experiments designed to study the electrically increase heart rate (HR; neurogenic tachycardia) or blood pressure (BP; neurogenic vasopressor response), respectively. Then rats were artificially ventilated with air (10 ml/kg; 60 strokes/min) by using the respiratory system (7025 rodent ventilator; Ugo Basile, Comerio, Italy). Both vagus nerves were cut in their cervical segment. Mean BP (MBP), systolic BP (SBP), and diastolic BP (DBP) were measured from the right carotid artery via a transducer (ISOTEC; Hugo Sachs Elektronik-Harvard Apparatus GmbH). HR was recorded from the ECG by means of subcutaneous electrodes. Body temperature was maintained constant at approximately 36 to 37°C by using a heating pad (Bio-Sys-Tech, Białystok, Poland) and monitored by a rectal probe transducer (Physitemp BAT10; Physitemp Instruments, Inc., Clifton, NJ). The left femoral vein was cannulated for intravenous administration of drugs. Most substances were administered intravenously in a volume of 0.5 ml/kg. Pentobarbitone, atropine, 4-nitrophenyl-4-(dibenzofuranyl)[[(1,3]dioxol-5-yl)(hydroxy)methyl]pyperidine-1-carboxylate (JZL184), and 3-‘[aminocarboxyl][1,1’-biphenyl]-3-yl]-cyclohexylcarbamate (URB597) were given intraperitoneally in a volume of 2 ml/kg. The right femoral vein was prepared for infusion of vasopressin (VP) or prostaglandin F2α (PGE2) by means of a Graseby 3100 syringe pump (Graseby Medical, Watford, Herts., UK). After 15 to 30 min of equilibration, during which the cardiovascular parameters were allowed to stabilize, experiments were performed.

**Experimental Protocol.** The experimental protocol is shown in Fig. 1. Each animal received an injection of pancuronium (0.8 μmol/kg i.v.) to avoid twitches associated with electrical stimulation. Pancuronium was also administered to those rats in which an increase in HR or BP was induced chemically by isoprenaline (ISO) and NA to ensure identical experimental conditions. Five minutes later the first stimulus (S1) was applied. In some experiments, increases in HR and BP were induced by the administration of ISO (0.1 nmol/kg) and NA (1 nmol/kg), respectively. In other preparations, increases in HR and BP were elicited by electrical stimulation of the preganglionic sympathetic nerve fibers; an electrical field was generated between the respective pithing rod in the vertebral column and an indifferent electrode was placed ventrally. The parameters of electrical stimulation, delivered from a Stimulator T (Hugo Sachs Elektronik-Harvard Apparatus GmbH), were 0.75 Hz, 1 ms, 50 V, and 5 or 15 pulses for increases in HR or BP, respectively. We chose doses of ISO and NA that caused an increase in HR and BP of comparable magnitude to that induced by electrical stimulation. Five minutes after S1 MI was induced by opening the chest and after left coronary artery ligation with a monofilament suture (Ethicon Endo-Surgery, Cincinnati, OH), as described previously (Pfeffer et al., 1985). In control animals the chest was opened, and the pericardial sac was torn (sham operation). To prevent the MI-mediated fall in baseline diastolic blood pressure, an infusion of VP or PGE2, was started 2 min before MI at a rate of 0.6 to 6.1 IU/kg/h or 0.08 to 0.7 mg/kg/h, respectively. Three subsequent stimuli (S2, S3, and S4)
were administered at 10-min intervals after the induction of MI or sham operation.

The effect of MI or sham operation was also examined after the administration of receptor antagonists, enzyme blockers, or their respective vehicles: CB1, rimonabant (0.1 μmol/kg; regimen described for the pithed rat; Malinowska et al., 2001); CB2, N-(15S)-endo-1,3,3-trimethyl-bicyclo[2.2.1]heptan-2-yl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528) (3 μmol/kg; regimen described for the rat in vivo; Rinaldi-Carmona et al., 1998); fatty acid amide hydrolase (FAAH), URB597 (1 μmol/kg; regimen described for the mouse in vivo; Fegley et al., 2005); and monoenoylglycerol lipase (MAGL), JZL184 (10 μmol/kg; regimen described for the mouse in vivo; Kinsey et al., 2011). The latter drugs were administered as described in Fig. 1. In one series of experiments, rimonabant (0.1 μmol/kg) was given 5 min after MI. Because the effect of the TRPV1 receptor antagonist capsazepine (1 μmol/kg) was short-lived, this drug was given intravenously twice, 2 min before S1 and again 2 min before S2 or S3 (regimen described for the anesthetized rat; Lupinski et al., 2011). Identical time schedules were used for the experiments with chemical and electrical stimulation.

After the experiments, the ischemic area was verified with Evans blue dye (1 mL; 2%; intravenously). Hearts were excised, frozen, and cut into thin slices, and then the ischemic area was determined with a stereoscopic microscope equipped with a camera. Experiments with an ischemic area of less than 20% of the left ventricle were excluded.

**Data Analysis.** Results are given as means ± S.E.M. (n = number of animals). To quantify the effect of MI on the electrically, ISO-, or NA-induced increase in HR or BP, the ratios S2/S1, S3/S1 and S4/S1 were determined. These ratios were expressed as percentages of the respective ratios obtained from the sham-operated animals. To define the receptors involved in the inhibitory action of MI, analogous ratios were determined in the presence of the respective receptor antagonists. For comparison of mean values, Student’s t test for unpaired data were used. When two or more treatment groups were compared with the same control, the one-way analysis of variance followed by Dunnett’s test was used. Survival rates were compared with Gehan-Wilcoxon and Kaplan-Meier survival curves. Differences were considered significant when P < 0.05.

**Materials.** Capsazepine (Tocris Bioscience, Bristol, UK), JZL184 (Cayman Chemical, Ann Arbor, MI), URB597 (Cayman Chemical), rimonabant (sano-aventis, Bridgewater, NJ), SR144528 (sano-aventis), (∞)-noradrenaline bitartrate (Sigma-Aldrich, Munich, Germany), (-)-isoprotrotenol hydrochloride (Sigma-Aldrich), pancuronium dibromide (Sigma-Aldrich), [Lys4]-vasopressin (Sigma-Aldrich), prostaglandin F2α, tris salt (Sigma-Aldrich), and pentobarbital sodium (pentobarbital) (Biowet, Pulawy, Poland) were used in the experiments. Pancuronium and PGF2α were dissolved in isotonic saline. Vasopressin was provided by the manufacturer (Sigma-Aldrich) as an aqueous solution (18.2 IU/ml), which was diluted (1:13.5) in isotonic saline before the experiment. Noradrenaline was dissolved in saline containing 6 mM acetic acid. JZL184 was dissolved in dimethyl sulfoxide (DMSO). URB597 was dissolved in a mixture of DMSO and Tween 80 (1:1) and then diluted (3:7) in isotonic saline immediately before the experiment. A stock solution of rimonabant was prepared in a mixture of ethanol and Cremophor El (1:1) and further diluted (1:9) in isotonic saline immediately before the experiment. A stock solution of SR144528 was prepared in a mixture of DMSO, ethanol, and Cremophor El (2:1:1) and further diluted (1:10) in isotonic saline immediately before the experiment. Capsazepine was first dissolved in DMSO and further diluted (1:10) in isotonic saline containing 10% Tween 80 and 10% ethanol. The solvents did not affect basal cardiovascular parameters with the following exceptions. The solvent for capsazepine produced a short-lived (20–30 s) decrease in HR (by approximately 10–15 beats/min) and a slight increase in DBP (by approximately 3–5 mm Hg). HR and DBP returned to baseline within 2 min. The nomenclature used to identify receptors conforms to the guidelines of the Committee on Receptor Nomenclature and Drug Classification of the International Union of Basic and Clinical Pharmacology (Pertwee et al., 2010).

**Results**

**General.** Basal HR and DBP, measured immediately before S1 in control (sham-operated) rats are given in Tables 1 and 2. With one exception (JZL184), the antagonists/enzyme blockers under study did not affect the latter parameters (Tables 1 and 2). The antagonists/enzyme blockers also did not modify basal SBP and MBP, which in control rats amounted to 72.8 ± 1.4 and 54.9 ± 1.0 mm Hg (n = 31), respectively (basal values related to the experiments shown in Fig. 2B). Because basal HR (but not basal DBP; Table 2) before S1 in the presence of JZL184 was significantly lower than the respective value in control rats not exposed to this enzyme blocker [271.2 ± 4.5 beats/min (n = 17) versus 300.3 ± 6.4 beats/min (n = 17), respectively; P < 0.001] we did not examine the influence of JZL184 on the neurogenic tachycardia.

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**Fig. 1.** Experimental protocol used to examine the influence of MI on the electrically or chemically induced increase in HR or BP in pithed and vagotomized rats. Each animal received an injection of pancuronium (0.8 μmol/kg) 5 min before the first stimulus (S1) was applied. HR and BP were increased by bolus injection of isoprenaline (0.1 nmol/kg) or noradrenaline (1 nmol/kg), respectively, or electrical stimulation (0.75 Hz; 1 ms; 50 V; 5 or 15 pulses for increases in HR or BP, respectively) of the preganglionic sympathetic nerve fibers innervating heart or blood vessels. Left coronary artery ligation or sham operation was performed 5 min after S1. An infusion of VP or PGF2α start 2 min before MI. Stimulations (S2–S4) were repeated at intervals of 10 min after the induction of MI or sham operation. Interacting drugs were injected as follows (relative to S1): capsazepine, 1 μmol/kg, −2 min because of its short-lived effect administration was repeated 2 min before S2 (increase in HR) or S3 (increase in BP); rimonabant, 0.1 μmol/kg or SR144528, 3 μmol/kg, −10 min (rimonabant was injected 10 min after S1 in an additional experimental series; not shown); URB597, 1 μmol/kg, −35 min (not shown); and JZL184, 10 μmol/kg, −115 min (not shown). Drugs were administered intravenously, except URB597 and JZL184, which were given intraperitoneally.
Changes in heart rate induced electrically or chemically in pithed and vagotomized rats with sham operation

Up to four stimuli (S1–S4) were administered to pithed rats. An increase in heart rate was induced by electrical stimulation of the preganglionic sympathetic nerve fibers innervating the rat heart or by bolus injection of isoprenaline. Rimonabant was given 10 min before or 10 min after S1, SR144528 was administered 10 min prior to S1, and capsazepine (1 μmol/kg) was administered 2 min before S1 and S2. URB597 was given 35 and 115 min before S1, respectively. For the experimental protocol, see Fig. 1. The changes in HR are shown either as absolute values (for S1) or as ratios (relative to S1) for the three subsequent stimuli (S2, S3, and S4). Mean ± S.E.M.

<table>
<thead>
<tr>
<th>Type of Stimulation</th>
<th>Antagonist</th>
<th>Dose (μmol/kg)</th>
<th>n</th>
<th>Basal HR Before S1 (beats/min)</th>
<th>Induced by S1</th>
<th>S2/S1</th>
<th>S3/S1</th>
<th>S4/S1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrical 50 V, 0.75 Hz, 1 ms, 5 pulses</td>
<td>No</td>
<td>6</td>
<td>330.5 ± 9.5</td>
<td>53.8 ± 5.8</td>
<td>0.98 ± 0.06</td>
<td>1.00 ± 0.04</td>
<td>1.00 ± 0.10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rimonabant (before sham operation)</td>
<td>0.1</td>
<td>7</td>
<td>336.0 ± 12.1</td>
<td>58.5 ± 6.0</td>
<td>0.90 ± 0.05</td>
<td>0.91 ± 0.03</td>
<td>0.91 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Rimonabant (after sham operation)</td>
<td>0.1</td>
<td>6</td>
<td>307.2 ± 5.8</td>
<td>49.8 ± 5.5</td>
<td>0.94 ± 0.08</td>
<td>1.04 ± 0.04</td>
<td>0.98 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>SR144528</td>
<td>3</td>
<td>5</td>
<td>326.0 ± 7.2</td>
<td>41.2 ± 7.4</td>
<td>0.97 ± 0.06</td>
<td>0.96 ± 0.06</td>
<td>0.96 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Capsazepine</td>
<td>1</td>
<td>5</td>
<td>319.8 ± 8.9</td>
<td>49.8 ± 8.0</td>
<td>0.83 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>URB597</td>
<td>1</td>
<td>5</td>
<td>305.8 ± 5.7</td>
<td>43.0 ± 7.1</td>
<td>0.94 ± 0.02</td>
<td>0.97 ± 0.05</td>
<td>1.06 ± 0.06</td>
</tr>
<tr>
<td>Isoprenaline 0.1 nmol/kg</td>
<td>No</td>
<td>4</td>
<td>327.0 ± 1.5</td>
<td>52.8 ± 10.0</td>
<td>1.11 ± 0.10</td>
<td>0.93 ± 0.13</td>
<td>0.94 ± 0.10</td>
<td></td>
</tr>
</tbody>
</table>

Changes in diastolic blood pressure induced electrically or chemically in pithed and vagotomized rats with sham operation

Up to four stimuli (S1–S4) were administered to pithed rats. An increase in diastolic blood pressure was induced by electrical stimulation of the preganglionic sympathetic nerve fibers innervating the rat heart or by bolus injection of isoprenaline. Rimonabant was given 2 min before S1 and S3. URB597 and JZL184 were given 35 and 115 min before S1, respectively. For the experimental protocol, see Fig. 1. The changes in DBP are shown either as absolute values (for S1) or as ratios (relative to S1) for the three subsequent stimuli (S2, S3, and S4). Mean ± S.E.M.

<table>
<thead>
<tr>
<th>Type of Stimulation</th>
<th>Antagonist</th>
<th>Dose (μmol/kg)</th>
<th>n</th>
<th>Basal DBP Before S1 (mm Hg)</th>
<th>Induced by S1</th>
<th>S2/S1</th>
<th>S3/S1</th>
<th>S4/S1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrical 50 V, 0.75 Hz, 1 ms, 15 pulses</td>
<td>No</td>
<td>6</td>
<td>44.2 ± 2.5</td>
<td>37.8 ± 4.7</td>
<td>0.96 ± 0.04</td>
<td>0.94 ± 0.05</td>
<td>0.98 ± 0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rimonabant (before sham operation)</td>
<td>0.1</td>
<td>6</td>
<td>44.3 ± 2.4</td>
<td>41.0 ± 3.0</td>
<td>0.70 ± 0.05</td>
<td>0.76 ± 0.04</td>
<td>0.76 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>SR144528</td>
<td>3</td>
<td>6</td>
<td>46.2 ± 2.1</td>
<td>40.7 ± 5.1</td>
<td>0.97 ± 0.02</td>
<td>0.95 ± 0.03</td>
<td>0.89 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Capsazepine</td>
<td>1</td>
<td>5</td>
<td>50.0 ± 4.8</td>
<td>43.1 ± 2.7</td>
<td>—</td>
<td>0.95 ± 0.04</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>URB597</td>
<td>1</td>
<td>8</td>
<td>44.0 ± 1.6</td>
<td>42.9 ± 3.8</td>
<td>0.90 ± 0.04</td>
<td>0.91 ± 0.05</td>
<td>0.88 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>JZL184</td>
<td>10</td>
<td>8</td>
<td>40.0 ± 1.0</td>
<td>35.0 ± 1.6</td>
<td>0.93 ± 0.03</td>
<td>0.95 ± 0.03</td>
<td>0.94 ± 0.04</td>
</tr>
<tr>
<td>Noradrenaline 1 nmol/kg</td>
<td>No</td>
<td>5</td>
<td>54.5 ± 2.9</td>
<td>36.1 ± 2.7</td>
<td>0.96 ± 0.05</td>
<td>0.99 ± 0.04</td>
<td>1.01 ± 0.04</td>
<td></td>
</tr>
</tbody>
</table>

Influence of MI on the electrically or catecholamine-induced increase in HR (A) and SBP, MBP, and DBP (B and C). For electrical stimulation (ES), the preganglionic sympathetic nerve fibers were stimulated by 5 or 15 pulses (for increases in HR or BP, respectively) of 50 V and 1 ms at 0.75 Hz. In separate experimental series, ISO (0.1 nmol/kg) or NA (1 nmol/kg) was injected intravenously to increase HR or BP, respectively. To compensate for the MI-induced decrease in blood pressure, VP or PGF2α was infused intravenously. Mean ± S.E.M. of three to six rats is shown. *, P < 0.05; **, P < 0.01; †††, P < 0.001 compared with the sham-operated rats.

Electrical stimulation (0.75 Hz; 1 ms; 50 V; five pulses) of the preganglionic sympathetic nerve fibers innervating the rat heart or injection of ISO (0.1 nmol/kg) increased HR during S1 by approximately 50 beats/min (Table 1). Electrical stimulation (0.75 Hz; 1 ms; 50 V; 15 pulses) of the preganglionic sympathetic nerve fibers innervating blood vessels or injection of NA (1 nmol/kg) increased DBP, SBP, and MBP during S1 by approximately 40 mm Hg (Table 2; data not shown). The degree of the tachycardia and vasopressor responses did not markedly change upon repeated electrical or chemical stimulation (S2–S4); in other words, the ratios S2/S1, S3/S1, and S4/S1, were close to or slightly lower than unity (Tables 1 and 2; data not shown). Rimonabant (0.1 μmol/kg), SR144528 (3 μmol/kg), capsazepine (1 μmol/kg), URB597 (1 μmol/kg), and JZL184 (10 μmol/kg) and their vehicles did not affect the neurogenic tachycardia and vasopressor responses by themselves (see S1 values in Tables 1 and 2; data not shown).

Influence of Myocardial Infarction on Basal HR and BP. Induction of MI evoked a slight rise in heart rate by approximately 15 beats/min, which lasted for approximately 5 to 10 min, and a profound hypotension, which developed...
ments in which changes in electrically stimulated increases in HR, MI even increased the electrically stimulated increase in SBP or MBP was approximately 25%. Thus, in all further experiments we have focused on changes in HR and DBP. Moreover, in contrast to the changes in HR, MI even increased the electrically stimulated increase in SBP by approximately 20% when VP was used as vasoconstrictor (in the case of DBP significant 10 min after MI; Fig. 2C; data not shown). So, all further experiments were dedicated to the examination of the influence of MI on the electrically stimulated increase in HR and DBP after MI were studied.

In contrast to the neurogenic tachycardic and vasopressor responses, MI did not affect the ISO-stimulated (Fig. 2A) and NA-stimulated (Fig. 2C) increases in HR and DBP, respectively. The CB₁ receptor antagonist rimonabant (given 10 min before MI) effectively prevented the inhibitory effect of MI throughout the experiments (up to 30 min after induction of the MI; Fig. 3). In additional experiments, the same dose of rimonabant, but injected 5 min after MI, partially diminished the inhibitory effect of MI on the neurogenic tachycardia (Fig. 3A); the strongest effect was observed at 30 min after induction of MI. Unlike the CB₁ receptor antagonist rimonabant, the CB₂ receptor antagonist SR144528 and the TRPV1 receptor antagonist capsazepine (Fig. 3) failed to influence the inhibitory effect of MI on the electrically stimulated increases in HR and DBP. Because of the short-lived effect of capsazepine its effect could be examined only once after the induction of MI.

The FAAH inhibitor URB597 (1 μmol/kg) and the MAGL inhibitor JZL184 (10 μmol/kg) tended to increase the inhibitory effect of MI on the neurogenic vasopressor response (Fig. 4). The effect became significant for URB597 and JZL184 30 and 20 min, respectively, after coronary artery ligation and amounted to 17% in each case (Fig. 4, B and C). By contrast, URB597 did not affect the MI-induced inhibition of the electrically induced increase in HR (Fig. 4A); for the reason given above, JZL184 could not be examined in this experimental setting.

**Influence of Antagonists and Enzyme Inhibitors on Ischemic Area Size and Survival Rate.** The ischemic area (percentage of left ventricle volume) induced by left coronary artery ligation in rats not injected with antagonists/ enzyme inhibitors was approximately 35% (Fig. 5A). The antagonists and enzyme inhibitors did not influence the ischemic area (Fig. 5A). We did not observe differences between rats treated with the group not receiving any antagonist.

![Fig. 3](https://example.com/fig3.png)

**Fig. 3.** Influence of the CB₁ receptor antagonist rimonabant (0.1 μmol/kg i.v.), the CB₂ receptor antagonist SR144528 (3 μmol/kg i.v.), or the TRPV1 receptor antagonist capsazepine (1 μmol/kg i.v.) on the inhibitory effect of MI on the electrically induced increase in HR (A) and DBP (B) in pithed and vagotomized rats. Rimonabant was administered 10 min before S₁ or 5 min after MI induction. SR144528 was given 10 min before S₁. Capsazepine was given 2 min before S₁ and S₂. Mean ± S.E.M. of three to six rats is shown. *, P < 0.05; **, P < 0.01; ***, P < 0.001 compared with the sham-operated rats. ∆, P < 0.05; ∆∆, P < 0.01; ∆∆∆, P < 0.001 compared with the group not receiving any antagonist.

![Fig. 4](https://example.com/fig4.png)

**Fig. 4.** Influence of inhibitors of the fatty acid amide hydrolase URB597 (A and B) and monoacylglycerol lipase JZL184 (C) on the inhibitory effect of myocardial infarction on the electrically induced increase in HR (A and DBP (B and C) in pithed and vagotomized rats. URB597 and JZL184 were given intraperitoneally 35 and 115 min before S₁, respectively. Mean ± S.E.M. of 3 to 10 rats is shown. *, P < 0.05; **, P < 0.01; ***, P < 0.001 compared with the sham-operated rats. ∆, P < 0.05; ∆∆, P < 0.01 compared with the group not receiving any enzyme inhibitor.
with VP or PGF$_{2\alpha}$; for the untreated groups the ischemic area was $35.8 \pm 3.0\%$ ($n = 10$) and $37.4 \pm 3.9\%$ ($n = 11$), respectively. For this reason, cumulated values are given.

The survival rate of rats that were not exposed to an antagonist/enzyme blocker slightly decreased after MI induction and was 84% at the end of the experiments (30 min after MI induction; $n = 31$) (Fig. 5B). The CB$_1$ receptor blockade by rimonabant (0.1 $\mu$mol/kg; $n = 18$) given before MI markedly reduced survival to 55% 30 min after MI induction (Fig. 5B).

Rimonabant given after MI and the other drugs under study did not influence mortality (data not shown).

**Discussion**

The aim of the present study was to examine whether acute experimental MI affects the sympathetic transmission to vessels and the heart of pithed rats via a presynaptic mechanism. Because an inhibitory effect of MI occurred, we studied which types of cannabinoid or cannabinoid-like receptors are involved and whether the effect can be increased by the blockade of endocannabinoid-degrading enzymes. Rats were anesthetized with pentobarbitone, which, unlike urethane, does not attenuate the function of presynaptic receptors (Kurz et al., 2009). The electrically increased increases in HR and BP studied in pithed rats are associated predominantly with catecholamines released from the neuronal sympathetic nerve endings of the heart and vessels (data not shown). Our previous study allows us to exclude the possibility that catecholamines released from the adrenal medulla contributed to the neurogenic cardiovascular responses (Malinowska et al., 2001).

We used the pithed rat because, in this model, first the reflex tachycardia encountered in cardiogenic shock does not occur and the effects of drugs involve only peripheral sites. Cannabinoids can exert centrally mediated cardiovascular effects opposite in direction to those occurring peripherally, and the central effects can override the peripheral ones. This might explain why marijuana can aggravate MI (Mittleman et al., 2001; Caldicott et al., 2005; Mukamal et al., 2008; Tormey, 2012) although cannabinoids may have a local cardioprotective effect (Wagner et al., 2001; Liao et al., 2011). Second, pithing diminishes arrhythmias after coronary occlusion (Curtis et al., 1985). In our hands, MI produced arrhythmia frequently, which, however, appeared almost exclusively between 5 and 15 min after coronary artery ligation. We applied a model of acute myocardial ischemia because the levels of AEA and 2-AG in rat blood platelets and monocytes are particularly high within the initial 30 min after induction of MI (Wagner et al., 2001) and the elevated risk of triggering of MI by smoking cannabis is limited to the first 2 h after smoking (Tormey, 2012). In addition, during brief periods of MI in the rat isolated heart, noradrenaline is released mainly via the exocytotic pathway sensitive to presynaptic regulation. By contrast, with prolonged periods of ischemia nonexocytotic noradrenaline release predominates (Schömig et al., 1991; Kübler and Strasser, 1994). Because ligation of the left coronary artery decreased baseline BP, a vasopressor agent (VP or PGF$_{2\alpha}$) was routinely infused.

Ligation of the left coronary artery reduced the electrically stimulated increase in HR by 30 to 40% in animals undergoing infusion with VP or PGF$_{2\alpha}$. An inhibitory effect of MI (maximally by 35%) was also found with respect to the neurogenic vasopressor response in rats treated with PGF$_{2\alpha}$. By contrast, when vasopressin was used we obtained an enhancement of the electrically stimulated rise in DBP by 20%. This phenomenon might mean that VP overrides the inhibitory effect of MI by activation of facilitatory presynaptic V$_1$ receptors, which have been identified previously in the resistance vessels of pithed rats (Streefkerk et al., 2002). So we applied PGF$_{2\alpha}$ for the experiments in which the electrically induced vasopressor response was studied, which does not modify the sympathetic transmitter release (Hoang et al., 2003) or even decrease it at concentrations higher than those used here (Molderings et al., 1992). Moreover, we focused on the inhibition of the electrically induced rise in DBP rather than in MBP or SBP because the strongest signal was obtained with the former parameter. VP, which we would have preferred because it is released in shock associated with cardiovascular disorders (Hollenberg, 2011), was used for the experiments in which the electrically induced rise in HR was examined.

Is the inhibitory effect of MI on the electrically induced vasopressor and tachycardic responses connected to a presynaptic or postsynaptic mechanism? The experiments in which the electrically induced alterations in BP and HR were determined do not allow us to answer this question definitively. Thus, noradrenaline release was not examined directly but rather the postsynaptic signal elicited by endogenously released noradrenaline was determined. So, the possibility exists that the endocannabinoids released because of MI either affect noradrenaline release or interfered with the already released noradrenaline at the postsynaptic $\alpha$- or $\beta$-adrenoceptors, finally leading to an increase in BP and HR, respectively. Therefore, additional experiments were performed in which the effect of MI on the postsynaptic $\alpha$- or $\beta$-adrenoceptors, activated by exogenously added noradrenaline and isoprenaline, was considered. In the latter experiments, MI did not affect BP and HR compared with the rats with a sham operation. So, we can conclude that the inhibitory effect of MI on the electrically induced vasopressor and tachycardic responses is related to a site upstream of the
postsynaptic α- or β-adrenoceptors, i.e., to presynaptic CB1 receptors leading to an inhibition of exocytotic noradrenaline release. Our study does not allow us to determine whether this presynaptic receptor is located on the postganglionic and/or preganglionic sympathetic neuron.

Endogenous cannabinoids generated in monocytes and platelets and acting via CB1 receptors have been shown to contribute to the hypotension in acute experimental MI in anesthetized rats (Wagner et al., 2001). The involvement of endogenously formed cannabinoids acting via CB1 receptors is also suggested by the present study. Thus, the inhibitory influence of MI on the neurogenic tachycardic and vasopressor responses was counteracted by the CB1 receptor antagonist rimonabant. Usually, we applied rimonabant 5 min before induction of MI, and then it completely prevented the MI-stimulated reduction of the neurogenic tachycardic response. In one series the drug was administered 5 min after the induction of MI, and even in this case it partially antagonized the MI-associated depressant effect.

Endocannabinoids exert their cardioprotective influence via CB2 receptors (Lépèrier et al., 2006). In addition, AEA activates the TRPV1 receptor (Lupiński et al., 2011). However, in our model we can exclude the involvement of the above two receptors because the MI-induced inhibition of the neurogenic tachycardic and vasopressor responses was not modified by their antagonists, SR144528 and capsazepine, respectively.

To further corroborate the role of the endocannabinoids experiments were performed in which the degradation of the two major endocannabinoids was blocked. Degradation of AEA via FAAH was blocked by URB597, and degradation of 2-AG via MAGL was inhibited by JZL184. Both inhibitors increased the MI-induced inhibition of the neurogenic vasopressor effect, supporting a role for both of them in this pathophysiological mechanism.

With respect to the MI-related inhibition of the neurogenic tachycardia, we could not examine the role of endogenous 2-AG because the corresponding degradation blocker, JZL184, strongly reduced basal HR by itself. The FAAH blocker, URB597, did not further increase the MI-related inhibition of the neurogenic tachycardia. However, one should consider that a stable analog of AEA (methAEA) only slightly (by 20%) decreases the neurogenic tachycardia in pithed rats (Baranowska et al., 2008), suggesting that detection of a further increase is difficult. The weak or missing effect of endocannabinoid breakdown inhibitors might also be related to the fact that the endocannabinoid system has a ceiling effect in ischemia or there is already so much endocannabinoid in the biophase that synthetic adjucnts do not have a marked effect. In addition, the influence of 2-AG on the neurogenic vasopressor or tachycardia has so far not been studied in vivo. It is noteworthy that in vitro, 2-AG, unlike AEA, increased the noradrenaline release from the sympathetic nerve endings innervating the rat heart (Kurihara et al., 2001).

Stimulation of inhibitory presynaptic CB1 receptors during acute MI may diminish the negative effects of excessive noradrenaline release and this phenomenon may be cardioprotective. Does this effect also have a practical and, in particular, a prognostic relevance? We did not find changes in infarct size after administration of rimonabant or the enzyme inhibitors. Likewise, rimonabant, given 10 min before MI, failed to alter infarct size in a mouse model (Lim et al., 2009). On the other hand, rimonabant (given before MI) increased mortality in pithed (present study) and anesthetized rats (Wagner et al., 2001). The enzyme blockers did not improve survival in our study; one has, however, to consider that the survival rate had a high level in untreated rats. With respect to the detrimental effect of rimonabant on survival, mechanisms apart from the inhibition of excessive noradrenaline release may be implicated as well.

In conclusion, our results demonstrate that postganglionic and/or preganglionic sympathetic nerve fibers innervating the rat heart and resistance vessels serve as a target for endogenously formed cannabinoids released in the early phase of myocardial infarction. They inhibit the electrically evoked, neurogenic increases in heart rate and blood pressure via presynaptic CB1 receptors. Thus, the CB1 receptor-mediated inhibition of the excessive noradrenaline release from the sympathetic nerve fibers innervating the heart and vessels might play a protective role in myocardial ischemia.

This phenomenon even have a prognostic relevance because CB1 blockers decreased the survival rate. Functional presynaptic inhibitory CB1 receptors sensitive to AEA have also been identified in human heart (Molderings et al., 1999). Further studies are necessary to clarify whether drug therapies directed against presynaptic inhibitory CB1 receptor are effective in myocardial infarction.

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Authorship Contributions

Participated in research design: Rudz, Schlicker, and Malinowska.
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