



Acute myocardial ischemia enhances the vanilloid TRPV1 and serotonin 5-HT₃ receptor-mediated Bezold-Jarisch reflex in rats

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Abstract:

The Bezold-Jarisch reflex is characterized by a sudden bradycardia associated with hypotension induced by the activation of the vanilloid TRPV1 and serotonin 5-HT₃ receptors. This reflex is associated with several health conditions, including myocardial infarction. The aim of the present study was to elucidate the influence of acute experimental myocardial ischemia on the reflex bradycardia induced by anandamide and phenylbiguanide, agonists of the TRPV1 and 5-HT₃ receptors, respectively.

In urethane-anesthetized rats, the rapid *iv* injection of anandamide (0.6 μmol/kg) or phenylbiguanide (0.03 μmol/kg) decreased heart rate (HR) by about 7–10% of the basal values. Myocardial ischemia (MI) was induced by ligation of the left anterior coronary artery. The agonists were injected 5 min before MI (S₁) and 10, 20 and 30 min thereafter (S₂–S₄).

MI potentiated the anandamide-induced reflex bradycardia by approximately 105% at S₂ and 70% at S₃ but had no effect at S₄. This amplificatory effect of MI was virtually abolished by the TRPV1 receptor antagonist capsazepine (1 μmol/kg) and was not modified by the cannabinoid CB₁ receptor antagonist rimonabant (0.1 μmol/kg). MI also amplified the reflex bradycardia elicited by phenylbiguanide by approximately 110, 60 and 90% (S₂, S₃ and S₄, respectively), and this effect was sensitive to the 5-HT₃ receptor antagonist ondansetron (3 μmol/kg).

In conclusion, our results suggest that acute myocardial ischemia augments the Bezold-Jarisch reflex induced *via* activation of TRPV1 and 5-HT₃ receptors located on sensory vagal nerves in the heart.

Key words:

Bezold-Jarisch reflex, TRPV1 receptors, 5-HT₃ receptors, anandamide, phenylbiguanide, myocardial ischemia

Abbreviations: 5-HT – serotonin, AEA – anandamide, DBP – diastolic blood pressure, ECG – electrocardiogram, HR – heart rate, *ip* – intraperitoneal, *iv* – intravenous, MI – myocardial ischemia, OND – ondansetron, PBG – phenylbiguanide, PGF_{2α} – prostaglandin F_{2α}

Introduction

The Bezold-Jarisch reflex is an inhibitory cardiovascular reflex that originates in cardiac sensory receptors on the

vagal afferent pathways. Stimulation of these receptors, which are located primarily in the inferoposterior wall of the left ventricle, by stretch and a range of chemical substances (see below) increases parasympathetic activity and inhibits sympathetic activity, promoting profound reflex bradycardia, vasodilation and hypotension (for review, see [1, 3, 32, 33]). The Bezold-Jarisch reflex can be directly triggered by the activation of the serotonin 5-HT₃ receptors, the vanilloid TRPV1 receptors and the P2X purinoceptors located on vagal afferent nerve endings. The 5-HT₃ receptors are stimulated by serotonin, e.g., released from blood platelets, whereas the TRPV1 receptors are activated by the endogenous cannabinoid anandamide [21, 27] or by protons [9], and the P2X receptors are stimulated by ATP released from cells and nerve endings [25].

It has been suggested that the Bezold-Jarisch reflex plays a role in several clinical conditions, including coronary reperfusion, bradycardia and hypotension during coronary arteriography, syncope occurring as a result of exertion in aortic stenosis, vasovagal syncope and the elevation of sympathetic drive in heart failure (for review, see [3, 32]). A cardiodepressor reflex similar to the Bezold-Jarisch reflex is also suggested to occur in patients with inferoposterior but not anterior myocardial infarction (for review, see [3]). Thus, the vagal tone, which may be connected to the Bezold-Jarisch reflex, was found to be enhanced in patients with exercise-induced ischemia of the inferoposterior myocardium [14, 15]. It has also been shown that acute myocardial ischemia in anesthetized cats [11–13] and rabbits [10] increased myocardial interstitial acetylcholine levels both in ischemic and non-ischemic regions in a manner dependent on vagal innervation [12]. The ischemia-induced myocardial interstitial acetylcholine release was further increased by vagal stimulation [11]. The Bezold-Jarisch reflex induced by phenylbiguanide, a 5-HT₃ receptor agonist, increased myocardial interstitial acetylcholine levels in anesthetized cats [13]. Thus, it has been suggested that this reflex may play a role in the acetylcholine release elicited by brief ischemia in the heart [12]. However, there are only a few contradictory reports describing the influence of myocardial ischemia on the Bezold-Jarisch reflex. Acute myocardial infarction enhanced the ATP-induced Bezold-Jarisch reflex in rabbits [31]. However, the serotonin-elicited Bezold-Jarisch reflex was not affected by a one-day myocardial infarction, but it was impaired by long-term (30 day) myocardial ischemia in rats [26].

Myocardial infarction and unstable angina are known to lead to platelet activation and the production of various metabolites, including serotonin, protons [6] and anandamide [18, 35]. Thus, the aim of the present study was to examine the influence of acute myocardial ischemia on the Bezold-Jarisch reflex mediated *via* the 5-HT₃ and TRPV1 receptors in rats.

Material and Methods

General procedure

Adult male Wistar rats (weighing 180–350 g) were used in the study. The animals were housed in a room maintained at 22 ± 1°C under a 12 h light-dark cycle and had free access to water and rodent chow. All experiments were approved by the Local Ethics Committee in Białystok (Poland). Prior to the experiments, the rats were anesthetized with an intraperitoneal (*ip*) injection of urethane (14 mmol/kg). This anesthesia was sufficient until the end of experiments, and we did not observe any withdrawal reflexes elicited by paw-pinch. Following anesthesia, the animals were tracheotomized. The carotid artery was carefully separated from the vagus nerve and cannulated to measure the diastolic arterial blood pressure (DBP) *via* a pressure transducer (“ISOTEC”; Hugo Sachs Elektronik, March-Hugstetten, Germany). The heart rate (HR) was recorded using an electrocardiogram (EEG). The body temperature was kept constant at about 36–37°C using a heating pad (Bio-Sys-Tech, Białystok, Poland) and monitored with a rectal probe transducer (RDT 100; Bio-Sys-Tech, Białystok, Poland). The left femoral vein was cannulated for the intravenous (*iv*) administration of drugs at a volume of 0.5 ml/kg. The right femoral vein was prepared for the infusion of prostaglandin F_{2α} (PGF_{2α}) or saline by means of a Graseby 3100 syringe pump (Graseby Medical, Watford, Herts., UK). After 15–30 min of equilibration, during which the cardiovascular parameters were allowed to stabilize, the experiments were performed.

Experimental protocol

The Bezold-Jarisch reflex was evoked four times (S₁–S₄) *via* a rapid *iv* injection of anandamide

(0.6 $\mu\text{mol/kg}$) or phenylbiguanide (0.03 $\mu\text{mol/kg}$) using a protocol similar to our previous studies [7, 20, 21]. S_1 occurred before the left coronary artery ligation (myocardial ischemia, MI) or sham operation, and S_2 – S_4 occurred at 10 min intervals after the surgery. Because the reflex decreases in DBP (the hypotensive component of the Bezold-Jarisch reflex) induced by both agonists were too small to analyze the modificatory influence of MI, only the bradycardiac component of the reflex (i.e., the maximal decrease in HR induced by the agonist) was analyzed in detail. The left coronary artery ligation or sham operation was applied 5 min after S_1 . All experiments were initiated by an *iv* injection of the β_1/β_2 -adrenoceptor antagonist propranolol 10 min (in the majority of rats) or 12 min (animals that received rimonabant) before S_1 in order to stabilize HR at 350–370 beats/min in all experimental groups. Propranolol was also given 5 min after MI or sham operation to stabilize HR and avoid/diminish arrhythmias induced by the operation. The dose used (0.1–0.6 $\mu\text{mol/kg}$, *iv*) was dependent on the basal HR. Each animal also received two injections of pipecuronium 5 min before S_1 (0.6 $\mu\text{mol/kg}$, *iv*) and 5 min before S_3 (0.1 $\mu\text{mol/kg}$, *iv*) in order to block spontaneous breathing after thoracotomy. Immediately after the first dose of pipecuronium, artificial ventilation with air (10 ml/kg; 60 strokes/min) was initiated using a 7025 Rodent Ventilator (Hugo Sachs Elektronik, March-Hugstetten, Germany).

Myocardial infarction

Myocardial ischemia was induced by left coronary artery ligation with a 6.0 monofilament suture (Ethicon Endo-Surgery, Cincinnati, OH, USA) following a left thoracotomy, as previously described [35]. After the experiments, the ischemic area was verified with Evans blue dye (1 ml, 2%; *iv*). The hearts were excised, frozen and cut into thin slices (2 mm). The ischemic area (expressed as a percentage of left ventricle area) was determined using a stereoscopic microscope (Motic SMZ-143-N2GG, Xiamen, China) equipped with a digital camera. Experiments in which the ischemic area was less than 20% were excluded. In control animals, the chest was opened and the pericardium was cut open (sham operation). In order to prevent the MI-related fall in basal DBP, an infusion of $\text{PGF}_{2\alpha}$ at a rate of 0.05–1.3 $\mu\text{mol/kg/h}$ was initiated a short time before MI and maintained until the end of the experiment. Instead of $\text{PGF}_{2\alpha}$, the animals in the

sham groups received an infusion of isotonic NaCl solution. The effects of the MI or sham operation on the Bezold-Jarisch reflex were also examined following the administration of the CB_1 receptor antagonist rimonabant (0.1 $\mu\text{mol/kg}$), the TRPV1 receptor antagonist capsazepine (1 $\mu\text{mol/kg}$) or the 5-HT₃ receptor antagonist ondansetron (3 $\mu\text{mol/kg}$). Rimonabant and ondansetron were given 10 and 5 min before S_1 , respectively. Capsazepine, due to its short-lived effects, was injected 2 min prior to each stimulation, and because it increased mortality, all experiments ended after S_3 [20–22]. We excluded experiments in which we had difficulty in maintaining stable cardiovascular parameters due to too high arrhythmia after MI or a drastic fall in blood pressure (mainly experiments with the use of capsazepine and ondansetron; for further details, see “Influence of MI on survival rate” in the Results section).

Calculations and statistics

Results are given as the means \pm SEM (n = number of animals). The decrease in HR was calculated as % of the basal heart rate immediately before the injection of anandamide or phenylbiguanide. To quantify the effect of myocardial infarction/sham operation on the anandamide/phenylbiguanide-induced bradycardia, the ratios S_2/S_1 , S_3/S_1 and S_4/S_1 were determined and expressed in percentages. To define the receptor(s), analogous ratios were determined in the presence of the respective receptor antagonists. For the comparison of mean values, Student's *t*-test was used for paired (to examine the influence of MI and sham operation on S_1 and the possible effect of a particular antagonist on basal HR and DBP) or unpaired (for comparison between particular groups) data. When two or more treatment groups were compared to the same control, a one-way analysis of variance (ANOVA) followed by the Dunnett test was used. Survival rates were compared with Gehan-Wilcoxon and Kaplan-Meier survival curves. Differences were considered as significant when $p < 0.05$.

Drugs

The drugs were obtained from the following sources: anandamide, capsazepine, ondansetron (ondansetron hydrochloride) and phenylbiguanide (1-phenylbiguanide) were from Tocris Cookson Inc. (Bristol, UK); rimonabant was from Sanofi Recherche (Montpellier, France); ure-

Tab. 1. Basal diastolic blood pressure (DBP), heart rate (HR) and alterations in HR induced by anandamide or phenylbiguanide in urethane-anesthetized rats

Agonist	Antagonist	Sham operation					Myocardial ischemia						
		n	Basal DBP before S ₁ (mm Hg)	Basal HR before S ₁ (beats/min)	S ₁ (% of basal HR)	n	Basal HR before S _n (beats/min)	n	Basal DBP before S ₁ (mm Hg)	Basal HR before S ₁ (beats/min)	S ₁ (% of basal HR)	n	Basal HR before S _n (beats/min)
Anandamide	–	8	63.6 ± 3.9	371.5 ± 6.3	6.9 ± 0.9	6	397.5 ± 20.8	9	63.0 ± 7.0	359.0 ± 6.1	7.4 ± 1.0	5	396.2 ± 13.6
	Capsazepine	7	76.9 ± 8.3	349.0 ± 12.5	5.7 ± 0.7	5	366.8 ± 8.1	7	68.7 ± 5.9	345.0 ± 9.5	6.4 ± 0.6	3	363.0 ± 17.9
	Rimonabant	7	72.9 ± 10.6	355.1 ± 6.1	7.6 ± 1.1	5	368.4 ± 12.0	7	70.3 ± 4.7	349.6 ± 9.8	8.5 ± 1.4	4	367.8 ± 12.4
Phenylbiguanide	–	7	65.4 ± 7.8	346.3 ± 6.1	10.0 ± 1.6	6	350.9 ± 3.7	8	58.1 ± 6.8	347.5 ± 12.0	10.7 ± 0.8	4	362.3 ± 19.4
	Ondansetron	5	54.0 ± 1.6	352.8 ± 21.5	5.3 ± 0.7*	5	374.2 ± 17.9	6	55.0 ± 2.6	348.7 ± 4.5	5.3 ± 1.2**	6	358.7 ± 10.9

A reflex bradycardia was induced four times (S₁–S₄) with an *iv* injection of anandamide (0.6 µmol/kg) or phenylbiguanide (0.03 µmol/kg). A sham operation or myocardial ischemia was administered 5 min after S₁ and 10 min before S₂. Rimonabant (1 µmol/kg), ondansetron (3 µmol/kg) or capsazepine (1 µmol/kg) were given 10, 5 or 2 min before S₁, respectively. Basal DBP and HR were determined immediately before S₁ or the final S_n. The final S_n was S₄ for the groups treated with rimonabant or without antagonists, S₃ for the group with capsazepine and S₂ for the group with ondansetron. Data are given as the means ± SEM of *n* experiments. * *p* < 0.05, ** *p* < 0.01 compared to the respective group without ondansetron

thane, propranolol, Cremophor EL (polyethoxylated castor oil), dimethyl sulfoxide (DMSO) and Tween-80 were from Sigma-Aldrich (Steinheim, Germany); pipercuronium bromide was from Gedeon Richter (Budapest, Hungary); and ethanol was from POCH (Gliwice, Poland).

Capsazepine was dissolved in a mixture of ethanol, Tween-80, DMSO and saline (1 : 1 : 1 : 9.5, vol. ratio). A stock solution of rimonabant was prepared in a mixture of DMSO and Cremophor EL, which was further diluted in saline immediately before the experiment (1 : 1 : 18, vol. ratio). Anandamide was purchased from Tocris Cookson as a 10.1 mg/ml emulsion in soya water/oil (1 : 4, v/v) and diluted in saline before the experiment. All other drugs were dissolved in saline. The solvents did not affect the basal DBP or HR.

Results

General

In urethane-anesthetized rats treated with propranolol (0.1–0.6 µmol/kg) and pipercuronium (0.6 µmol/kg), the basal HR and DBP before S₁ were approximately

360 beats/min and approximately 60 mmHg (Tab. 1). Intravenous administration of the CB₁ receptor antagonist rimonabant (0.1 µmol/kg), the TRPV1 receptor antagonist capsazepine (1 µmol/kg) and the 5-HT₃ receptor antagonist ondansetron (3 µmol/kg) did not modify these baseline cardiovascular parameters (Tab. 1).

Influence of MI on basal HR and DBP

The induction of MI evoked a slight tachycardia by about 15–40 beats/min, arrhythmia and profound hypotension, which developed within a few minutes and persisted throughout the experiment. A slight tachycardia and arrhythmia (weaker than in the MI group) also occurred in the sham-operated animals. To overcome the tachycardia and arrhythmia, propranolol (0.1–0.6 µmol/kg, *iv*) was given to MI and sham-operated animals (for original traces, see Fig. 1). To prevent a fall in blood pressure, an infusion of PGF_{2α} (0.05–1.3 µmol/kg/h) was initiated just before left coronary artery ligation and was continued until the end of the experiments. In the presence of propranolol and PGF_{2α} (MI group only), HR and DBP remained stable throughout the experiment in both the sham-operated and MI animals. Thus, the basal HR and

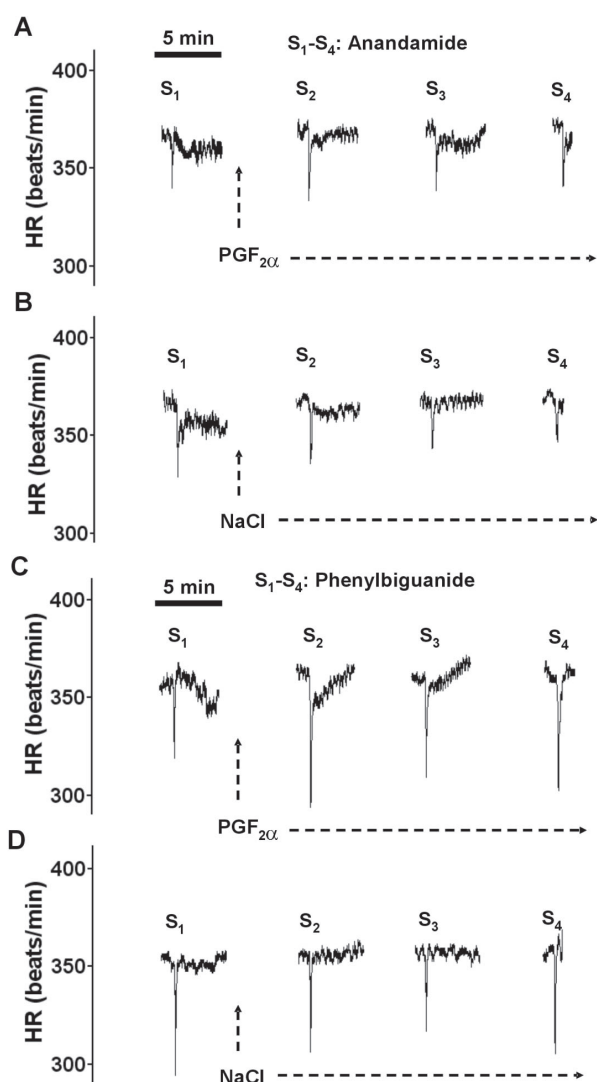


Fig. 1. Traces from representative experiments showing the influence of the sham operation (**B, D**) and myocardial ischemia (**A, C**; MI) on the Bezold-Jarisch reflex in urethane-anesthetized rats. The Bezold-Jarisch reflex was induced four times (S_1 – S_4) by the rapid intravenous injection (*iv*) of anandamide (**A, B**; 0.6 $\mu\text{mol/kg}$) or phenylbiguanide (**C, D**; 0.03 $\mu\text{mol/kg}$). A left coronary artery ligation (MI) or sham operation was applied 5 min after S_1 . Three subsequent injections of the agonist (S_2 , S_3 and S_4) were applied 10, 20 and 30 min after the induction of MI or sham operation. Each animal received an injection of propranolol (0.1–0.6 $\mu\text{mol/kg}$, *iv*) 15 or 17 min (for details, see Material and Methods) before and 5 min after MI or sham operation. Pípecuronium was given 5 min before S_1 (0.6 $\mu\text{mol/kg}$, *iv*) and 5 min before S_3 (0.1 $\mu\text{mol/kg}$, *iv*). An infusion of prostaglandin $F_{2\alpha}$ ($\text{PGF}_{2\alpha}$; 0.05–1.3 $\mu\text{mol/kg/h}$) was initiated immediately after MI. In sham-operated rats, saline (NaCl) was infused instead

DBP measured immediately before S_2 , S_3 and/or S_4 did not differ from the baseline values recorded before coronary artery ligation or sham operation (data not shown; for the final S_n , see Tab. 1; i.e., S_4 for the

groups without antagonists or treated with rimonabant and S_3 and S_2 for rats receiving capsazepine and ondansetron, because these experiments ended after S_3 and S_2 , respectively). We excluded experiments in which the MI-induced arrhythmia was too high.

Influence of MI on the anandamide-induced reflex bradycardia

The rapid *iv* injection of anandamide (0.6 $\mu\text{mol/kg}$) induced a short-term decrease in HR (the bradycardiac component of the Bezold-Jarisch reflex), which amounted to approximately 7% of basal values (S_1) before the sham operation or MI (Tab. 1). The three subsequent decreases in HR (S_2 – S_4) induced after the sham operation were lower than S_1 by about 20–30% (Fig. 2A). Left coronary ligation enhanced the reflex bradycardia induced by anandamide by 107 and 72% 10 and 20 min after the operation, respectively. Thirty minutes after MI, the anandamide-elicited decrease in HR was comparable to the S_4/S_1 values obtained in the sham-operated rats (Fig. 2A).

The TRPV1 receptor antagonist (capsazepine; 1 $\mu\text{mol/kg}$; Fig. 2B) and CB_1 receptor antagonist (rimonabant; 0.1 $\mu\text{mol/kg}$; Fig. 2C) did not modify the anandamide-induced reflex bradycardia in sham-operated animals (for S_1 , see Tab. 1; for S_2 – S_4 , see Fig. 2B and 2C). However, capsazepine completely prevented the amplificatory influence of myocardial ischemia on the anandamide-induced decreases in HR (Fig. 2B). On the other hand, rimonabant failed to modify the amplificatory effect of MI on the anandamide-induced reflex bradycardia (Fig. 2C).

Influence of MI on the phenylbiguanide-induced reflex bradycardia

The rapid *iv* injection of the 5-HT₃ receptor agonist phenylbiguanide (0.03 $\mu\text{mol/kg}$) induced a short-term decrease in HR that amounted to 10% of basal values (S_1) before the sham operation and left coronary ligation (Tab. 1). In sham-operated rats, the three subsequent decreases in HR elicited by this agonist (S_2 , S_3 and S_4) were reduced by about 20–30% in comparison to S_1 (Fig. 3A). MI potentiated the phenylbiguanide-stimulated reflex bradycardia by 103, 64 and 84% 10, 20 and 30 min after left coronary artery ligation, respectively (Fig. 3A).

The selective 5-HT₃ receptor antagonist ondansetron (3 $\mu\text{mol/kg}$) diminished the phenylbiguanide-

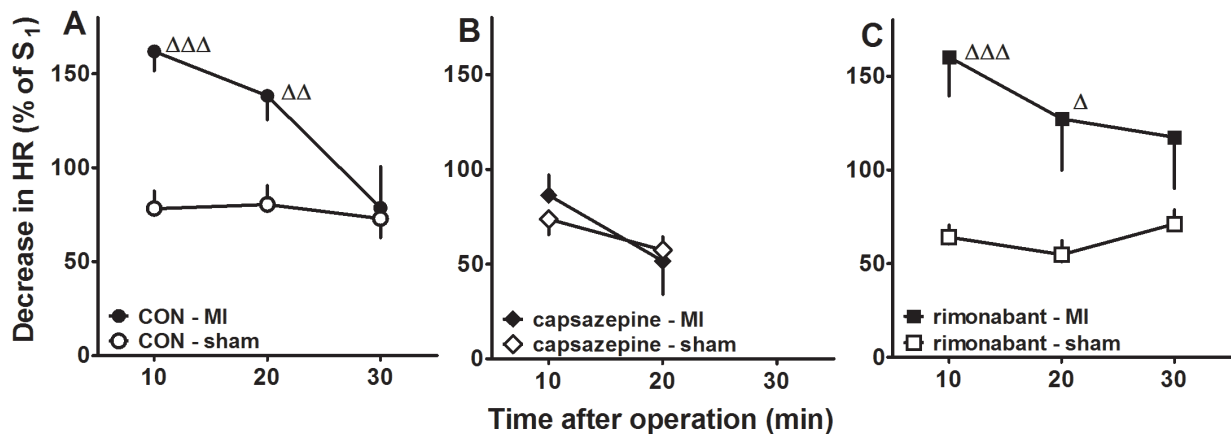


Fig. 2. Influence of myocardial ischemia (MI) on the decrease in heart rate (HR) induced by anandamide (0.6 $\mu\text{mol/kg}$) in urethane-anesthetized rats (A) and the influence of capsazepine (1 $\mu\text{mol/kg}$) (B) or rimonabant (0.1 $\mu\text{mol/kg}$) (C) on the amplificatory effect of MI. Anandamide was administered 5 min before (S_1) and 10, 20 and 30 min (S_2 – S_4) after the induction of MI or sham operation. The results are expressed as the percentages of S_1 . CON – control rats, which did not receive any antagonist. The data represent the means \pm SEM of 4–9 rats. Δ $p < 0.05$, $\Delta\Delta$ $p < 0.01$, $\Delta\Delta\Delta$ $p < 0.01$ compared to the respective value in the sham-operated rats

induced bradycardia (for S_1 values, see Tab. 1). Unfortunately, in the presence of ondansetron, only one stimulus could be administered after MI (S_2) because the blood pressure decreased too drastically to be overcome effectively by $\text{PGF}_{2\alpha}$ infusion. In addition, MI caused a strong arrhythmia, and only 50% of the rats survived until S_2 . Ondansetron completely prevented the amplification of the phenylbiguanide-induced bradycardia evoked by MI (Fig. 3B).

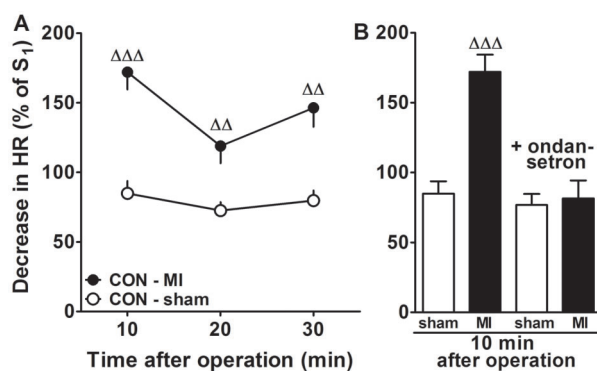


Fig. 3. Influence of myocardial ischemia (MI) on the decrease in heart rate (HR) induced by phenylbiguanide (0.03 $\mu\text{mol/kg}$) in urethane-anesthetized rats (A) and the effect of ondansetron (3 $\mu\text{mol/kg}$) on the amplificatory effect of myocardial ischemia (MI) (B). Phenylbiguanide was given 5 min before (S_1) and 10, 20 and 30 min (S_2 – S_4) after induction of MI or sham operation. The results are expressed as percentages of S_1 . CON – control rats, which did not receive ondansetron. The data represent the means \pm SEM of 5–8 rats. $\Delta\Delta$ $p < 0.01$, $\Delta\Delta\Delta$ $p < 0.001$ compared to the respective value in the sham-operated rats

Influence of MI on ischemic area size

The size of the ischemic area (expressed as a percentage of the left ventricle area) induced by left coronary artery ligation was approximately 35% and did not differ between the anandamide and phenylbiguanide group (in the absence of antagonists) (Fig. 4). The TRPV1 receptor antagonist capsazepine (1 $\mu\text{mol/kg}$) increased the ischemic area size by 31% compared to animals receiving anandamide only (Fig. 4). On the other hand, blockade of CB_1 receptors by rimonabant (0.1 $\mu\text{mol/kg}$) or of 5-HT_3 receptors by ondansetron (3 $\mu\text{g/kg}$) did not modify the size of the ischemic area in rats exposed to anandamide and phenylbiguanide, respectively (Fig. 4).

Influence of MI on survival rate

The survival rates were monitored every 5 min. In the group exposed to anandamide only, the survival rate was 100% over a time period of 15 min but then gradually decreased to 67% at the end of the experiment (Fig. 5). Rimonabant (0.1 $\mu\text{mol/kg}$) tended to increase the mortality rate (Fig. 5). Moreover, capsazepine (1 $\mu\text{mol/kg}$) decreased the survival rates to 71 and 43% 15 and 20 min after MI, respectively. Due to difficulties in maintaining stable cardiovascular parameters in rats receiving phenylbiguanide (with ondansetron), these experiments ended 10 min after MI. Therefore, an analysis of survival rates was not performed.

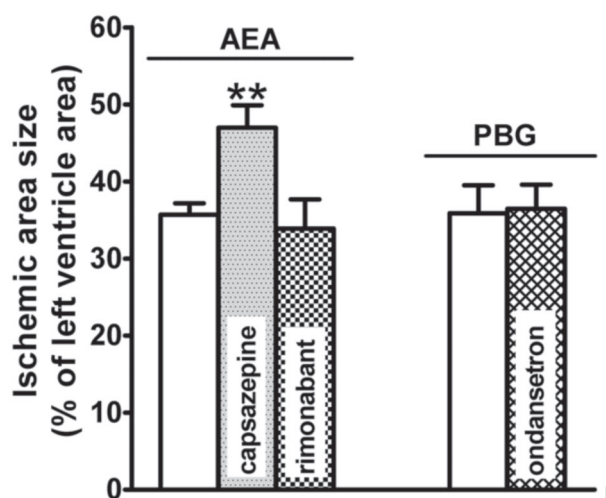


Fig. 4. Influence of capsazepine, rimonabant and ondansetron on the ischemic area size. Myocardial ischemia (MI) was induced using left coronary artery ligation in urethane-anesthetized rats. The Bezold-Jarisch reflex was evoked four times (S_1 5 min before MI and S_2 – S_4 10, 20 and 30 min after MI) by the injection of anandamide (AEA; 0.6 $\mu\text{mol/kg}$) or phenylbiguanide (PBG; 0.03 $\mu\text{mol/kg}$). Rimonabant (0.1 $\mu\text{mol/kg}$) or ondansetron (3 $\mu\text{mol/kg}$) were given 15 or 10 min before MI, respectively. Capsazepine (1 $\mu\text{mol/kg}$) was injected two minutes prior to each stimulation. The data represent the means \pm SEM of 6–8 rats. ** $p < 0.01$ compared to the corresponding group that did not receive antagonist

Discussion

The aim of the present study was to examine the influence of acute myocardial ischemia on the TRPV1 and 5-HT₃ receptor-mediated Bezold-Jarisch reflex in anesthetized rats. Animals were anesthetized with urethane because this anesthetic does not modify cardiovascular reflex responses [19]. Given that in urethane-anesthetized rats the basal HR is enhanced [19], propranolol was routinely used (i) to stabilize the basal HR at the level between 350–370 beats/min (because bradycardiac responses were more reproducible at this HR), (ii) to avoid/diminish arrhythmias induced by the MI or sham operation and (iii) to block cardiostimulatory reflex responses [6], which can be stimulated during ischemia by TRPV1 receptors located on cardiac afferent nerves [28]. Importantly, propranolol alone does not affect the Bezold-Jarisch reflex [7]. Pipecuronium was administered to allow for the artificial respiration needed for the MI/sham operated animals. As in our previous studies [22], PGF_{2 α} was infused to compensate for the marked de-

crease in the baseline DBP elicited by left coronary ligation. PGF_{2 α} does not modify sympathetic transmitter release [2] and, to the best of our knowledge, does not influence cardiovascular reflex responses. Sham-operated animals received an infusion of isotonic NaCl solution instead.

In order to induce the Bezold-Jarisch reflex *via* the TRPV1 receptors, we used anandamide, an endogenous cannabinoid that has been detected in the human heart [5] and shows increased levels in rats [35] and patients with acute MI [18]. To induce the Bezold-Jarisch reflex *via* the 5-HT₃ receptors, we used the specific, exogenous 5-HT₃ receptor agonist phenylbiguanide, but not its endogenous ligand serotonin, because the latter elicits cardiovascular responses *via* at least six types of receptors [30, 33]. Serotonin is increased in cats with brief myocardial ischemia and in patients during thrombosis-induced coronary artery occlusion [6]. Anandamide and phenylbiguanide were given at 0.6 and 0.03 $\mu\text{mol/kg}$, respectively. The hypotensive component of the Bezold-Jarisch reflex induced by such low doses of these agonists was too small to be examined in detail. Thus, only decreases in HR were quantified. Its amounted to 7–10% of basal values, similar to that obtained by Rocha et al. [31] in anesthetized rabbits, in which the P2X receptor-mediated Bezold-Jarisch reflex was elicited by ATP. We induced reflex bradycardia four times (S_1 – S_4), and the bradycardia elicited by S_2 , S_3 or S_4 was approximately 20–30% lower than S_1 , probably as a result of tachyphylaxis. As antagonists, capsazepine (1 $\mu\text{mol/kg}$) and ondansetron (3 $\mu\text{mol/kg}$) were used, which are known to strongly inhibit (by about 50%) the Bezold-Jarisch reflex induced by anandamide (2–3 $\mu\text{mol/kg}$) [21] and almost completely inhibit the reflex stimulated by phenylbiguanide (0.06 $\mu\text{mol/kg}$) [20]. In the present study, ondansetron markedly reduced the reflex bradycardia (S_1) elicited by phenylbiguanide, whereas capsazepine failed to modify the reflex bradycardia elicited by anandamide. The probable explanation for the latter phenomenon is that the data point for the decrease in HR induced by anandamide (0.6 $\mu\text{mol/kg}$) is localized on the first plateau of the dose-response curve for this compound (see Fig. 3 in [21]).

We found that acute experimental ligation of the left coronary artery strongly enhanced the reflex bradycardia elicited both by anandamide and phenylbiguanide. The amplificatory effect reached 100% ten minutes after ligation of the coronary artery with either agonist. This response remained fairly constant

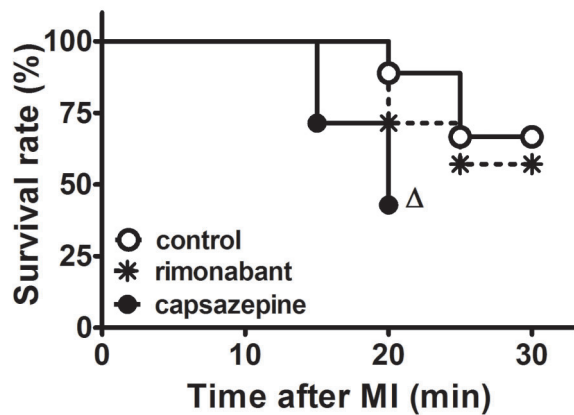


Fig. 5. Influence of capsazepine and rimonabant on the survival rate in urethane-anesthetized rats in which the effect of myocardial ischemia (MI; induced by left coronary artery ligation) on the Bezold-Jarisch reflex evoked four times (S_1 – S_4) by injection of anandamide (AEA; 0.6 $\mu\text{mol/kg}$) was examined. S_1 was applied 5 min before MI and S_2 – S_4 10, 20 and 30 min after MI. Rimonabant (0.1 $\mu\text{mol/kg}$) was given 15 min before MI. Capsazepine (1 $\mu\text{mol/kg}$) was injected two minutes prior to each stimulation. The data represent the means \pm SEM of 6–8 rats. ^Δ $p < 0.05$ compared to the corresponding group that did not receive antagonist

over 30 min (the time period chosen in the present study) with phenylbiguanide but returned to the basal level within this time period in the case of anandamide. The amplificatory influence of MI on the anandamide- and phenylbiguanide-elicited Bezold-Jarisch reflex was abolished by capsazepine and ondansetron, suggesting that the potentiating effect of acute MI on reflex bradycardia is mediated *via* the TRPV1 and 5-HT₃ receptors, respectively. We can exclude the possibility that the enhancement of the Bezold-Jarisch reflex is mediated through one receptor only because it has been previously demonstrated that anandamide and phenylbiguanide activate distinct receptor entities; i.e., anandamide activates the TRPV1 receptors, while phenylbiguanide activates the 5-HT₃ receptors located on the vagal afferent C-fibers in the heart [4, 20, 21]. Thus, our results are in line with the report by Rocha et al. [31], in which acute MI amplified the Bezold-Jarisch reflex induced by ATP *via* P2X receptor activation.

The endocannabinoid anandamide not only stimulates TRPV1 receptors [21], but it is also a CB₁ receptor agonist. In the heart, CB₁ receptors are located postsynaptically on heart muscle [34] and presynaptically on sympathetic nerve endings innervating the heart [23]. They decrease heart contractility and inhibit noradrenaline release from sympathetic nerve endings, respectively. The function of presynaptic

CB₁ receptors in the rat heart is diminished by acute MI [24]. Thus, we examined the influence of the CB₁ receptor antagonist rimonabant on the anandamide-stimulated Bezold-Jarisch reflex. However, rimonabant (used at 0.1 $\mu\text{mol/kg}$ as in our previous studies [22–24]) failed to modify the amplificatory influence of MI on the reflex bradycardia elicited by the endocannabinoid.

It has been postulated that TRPV1 receptors located on cardiac afferents may serve as a molecular detector of MI to activate cardiac nociceptors [28]. In addition, a beneficial cardioprotective influence of these receptors against cardiac injury has been demonstrated in isolated heart ([9]; for review, see [29]). Moreover, TRPV1 gene deletion increased mouse mortality 3 days after MI [9]. To the best of our knowledge, our report is the first to show that blockade of TRPV1 receptors increases ischemic area size and decreases the survival rate after acute MI. Thus, our present results confirm and extend the knowledge regarding the cardioprotective properties of TRPV1 receptors.

The 5-HT₃ receptor antagonist ondansetron did not affect MI size but strongly enhanced the cardiopressor effects of MI and shortened rat survival. Our observations are in line with the cardiotoxic effects of this compound described in patients [8]. The CB₁ receptor antagonist rimonabant tended to increase rat mortality without affecting MI size. In the study by Wagner et al. [35], administration of rimonabant before acute MI strongly reduced the rat survival rate without influencing MI size. However, the Authors of this study, used a 65-fold higher dose of rimonabant than we used in our study. In anesthetized mice, chronic, but not acute, administration of rimonabant reduced myocardial infarct size [17]. Our observation is important given that rimonabant is suggested as a new therapeutic approach for the treatment of obesity and cardiovascular risk factors [16].

In conclusion, our results suggest that acute myocardial ischemia causes a strong amplification of the Bezold-Jarisch reflex induced *via* activation of TRPV1 and 5-HT₃ receptors located on sensory vagal nerves in the heart. In addition, we demonstrated the cardioprotective effect of TRPV1 receptors against acute myocardial infarction. Given that the Bezold-Jarisch reflex plays a role in several clinical conditions, including myocardial ischemia, we cannot exclude the potential clinical significance of our present findings.

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