Excess ischemic tachyarrhythmias trigger protection against myocardial infarction in hypertensive rats

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ABSTRACT

Increased level of C-reactive protein (CRP) is a risk factor for cardiovascular diseases, including myocardial infarction and hypertension. Here, we analyze the effects of CRP overexpression on cardiac susceptibility to ischemia/reperfusion (I/R) injury in adult spontaneously hypertensive rats (SHR) expressing human CRP transgene (SHR-CRP). Using an in vivo model of coronary artery occlusion, we found that transgenic expression of CRP predisposed SHR-CRP to repeated and prolonged ventricular tachyarrhythmias. Excessive ischemic arrhythmias in SHR-CRP led to a significant reduction of infarct size (IS) compared with SHR. The proarrhythmic phenotype in SHR-CRP was associated with altered heart and plasma eicosanoids, myocardial composition of fatty acids in phospholipids, and autonomic nervous system imbalance before ischemia. To explain unexpected IS-limiting effect in SHR-CRP, we performed metabolomic analysis of plasma before and after ischemia. We also determined cardiac ischemic tolerance in hearts subjected to remote ischemic perconditioning (RIPer) and in hearts ex vivo. Acute ischemia in SHR-CRP markedly increased plasma levels of multiple potent cardioprotective molecules that could reduce IS at reperfusion. RIPer provided IS-limiting effect in SHR that was comparable with myocardial infarction observed in naive SHR-CRP. In hearts ex vivo, IS did not differ between the strains, suggesting that extra-cardiac factors play a crucial role in protection. Our study shows that transgenic expression of human CRP predisposes SHR-CRP to excess ischemic ventricular tachyarrhythmias associated with a drop of pump function that triggers myocardial salvage against lethal I/R injury likely mediated by protective substances released to blood from hypoxic organs and tissue at reperfusion.

Keywords: heart, myocardial infarction, ventricular arrhythmias, C-reactive protein, metabolomics, remote ischemic perconditioning
INTRODUCTION

The inflammatory response plays a crucial role in the pathophysiology of many cardiovascular disorders, including acute myocardial infarction and post-ischemic heart failure. Previous research has shown that C-reactive protein (CRP), a protein of the acute phase of inflammation, enhances the extent of myocardial damage associated with ischemic heart disease [1]. In humans, increased CRP production is a predictive marker for future coronary events, recurrent myocardial infarction, chronic heart failure, and cardiovascular death [2,3]. It has been shown that CRP, together with a CRP-activated complement, is deposited in atherosclerotic plaques and infarcted myocardium [4].

To investigate the effect of increased levels of CRP on pathogenetic mechanisms of cardiovascular diseases, various animal and experimental models have been used. In rodents, CRP is not an acute-phase reactant and their CRP does not activate complement [5]. Therefore, humanized CRP transgenic animals that activate rodent complement were created [6]. Transgenic expression of human CRP exacerbated the progression of post-ischemic heart failure in mice [7]. Similarly, antisense oligonucleotides designed to specifically hybridize to either rat or human CRP mRNA improved left ventricular systolic function in rats after myocardial infarction [8]. Finally, the administration of human CRP immediately after myocardial infarction activated complement, increased infarct size, and impaired the left ventricle (LV) systolic function in normotensive Wistar rats [6,9].

Recently, Pravenec et al. [10] created the humanized CRP transgenic rat strain SHR-CRP from spontaneously hypertensive rats (SHR). SHR is a widely studied rat model of human essential hypertension that is genetically predisposed to the development of the metabolic syndrome and characterized by an increased myocardial sensitivity to acute ischemia/reperfusion (I/R) injury [11-13]. In SHR-CRP, the overall concentration of CRP (endogenous rat and transgenic human) was markedly elevated compared to SHR controls. The increased CRP level was accompanied by multiple features of the metabolic syndrome including insulin resistance, hypertriglyceridemia and increased blood pressure [10,14,15]. Concerning the heart, the increased level of human CRP resulted in a perivascular accumulation of monocytes and oxidative stress in the LV of SHR-CRP [14,15]. However, the effect of transgenic expression of human CRP on myocardial I/R injury in hypertensive rats with the metabolic syndrome has not been determined.

In this study, we assessed the incidence and severity of ischemic and reperfusion ventricular arrhythmias and myocardial infarct size in hearts in vivo of transgenic SHR-CRP and SHR controls. Then, subsequent physiological, biochemical, and molecular biological analyses were performed to explain the observed changes in cardiac ischemic tolerance caused by the CRP transgene. Although counterintuitive, the results indicated that repeated and prolonged ventricular tachyarrhythmias occurring during coronary artery occlusion in SHR-CRP can trigger protective mechanisms that lead to infarct size reduction. Therefore, we revealed a novel link between ventricular arrhythmias and acute myocardial infarction caused by an I/R insult in hypertensive rats.
MATERIALS AND METHODS

Animal model

Adult male rats of SHR and SHR-CRP strains [16-20 weeks old, n=99 per strain, body weight (BW) 300-370 g] were used. Transgenic SHR-CRP were derived by microinjections of SHR fertilized ova with a construct containing cDNA for human CRP under control of the apoE promoter with the objective of driving expression of the CRP transgene in the liver where CRP is normally produced [10,15]. Rats were bred in the Institute of Physiology of the Czech Academy of Sciences, and housed in an air-conditioned animal facility and allowed free access to their standard chow and water. The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (published by the National Academy of Science, National Academy Press, Washington, D.C.). Experimental protocols were approved by the Animal Care and Use Committee of the Institute of Physiology, the Czech Academy of Sciences (Permit Number: 75/2016).

Myocardial I/R in vivo

Rats were subjected to acute I/R as described previously [16]. Briefly, anesthetized (sodium pentobarbital, 60 mg/kg i.p.; Sigma-Aldrich, Czech Republic) animals were intubated with a cannula connected to a rodent ventilator (Ugo Basile, Italy) and ventilated with room air at 68-70 strokes/min (tidal volume of 1.2 ml/100 g body weight). The rectal temperature was maintained between 36.5 and 37.5°C by a heated table throughout the experiment. Left thoracotomy was performed and a silk braided suture 5/0 (Chirmax, Czech Republic) was placed around the left anterior descending (LAD) coronary artery about 1–2 mm distal to its origin. Regional myocardial ischemia was induced after 15-min stabilisation by the tightening of the suture threaded through a polyethylene tube. After a 20-min occlusion period, the ligature was released and reperfusion of previously ischemic tissue continued. Chest was closed after 3 min of reperfusion, air was exhausted from thorax and spontaneously breathing animals were maintained in deep anaesthesia following 180 min by i.p. administration of pentobarbital (one third of the initial dose per hour). In the separate group of SHR, remote ischemic perconditioning (RIPer) was induced by three cycles of 4 min occlusion of both hindlimbs (by the tourniquet) followed by 4 min reperfusion starting 2 min after onset of ischemia. Dark-blue skin color was taken to indicate leg ischemia. A single-lead electrocardiogram (ECG) was registered during ischemia and the first 3 min of reperfusion.

Myocardial I/R ex vivo

Animals were anesthetized as above. Hearts were rapidly excised and perfused according to Langendorff under constant flow (adjusted to approximately 10 mL/min per g) as described [17] with non-recirculating Krebs–Henseleit solution containing (in mmol/L): NaCl 118.0; KCl 3.2; CaCl₂ 2.5; MgSO₄ 1.2; NaHCO₃ 25.0; KH₂PO₄ 1.2; glucose 7.0. The medium was saturated with 95% O₂ and 5% CO₂ (pH 7.4) and maintained at 37°C. Expected heart weights were calculated from the regression equations established on the basis of our previous data for heart-to-body weight ratio. Epicardial ECG were recorded with electrodes...
attached to the right atrium and the apex of the heart. After 20 min stabilization, regional 40 min no-flow ischemia was induced by occlusion of the LAD coronary artery followed by 60 min reperfusion. ECG was registered during ischemia and the first 10 min of reperfusion. In both in vivo and ex vivo hearts, rats exhibiting ventricular arrhythmias before I/R (during stabilization) were excluded from experiment.

**Infarct size determination and analysis of arrhythmias**

In open-chest rats, hearts were excised and washed with saline via aorta at the end of reperfusion. The area at risk (AR) was delineated by perfusion with 5% potassium permanganate in both experimental settings, as described earlier [16]. Frozen hearts were cut into slices 1 mm thick, stained with 1% 2,3,5-triphenyltetrazolium chloride (pH 7.4; 37°C; Sigma-Aldrich, Czech Republic) for 30 min and fixed in formaldehyde solution. Four days later, both sides of the slices were photographed. The IS, the size of AR and LV were determined by computerized planimetric method using the software Ellipse (ViDiTo, Slovakia). The incidence and severity of ventricular arrhythmias during the ischemic insult and at the beginning of reperfusion were analysed offline from ECG records by custom-designed software according to the Lambeth Conventions as previously described [18]. Briefly, premature ventricular complexes (PVCs) occurring as singles, salvos, or tachycardia (VT, a run of 4 or more consecutive PVCs) were counted separately. The incidence and the number of episodes of ventricular tachycardia (VT) and fibrillation (VF) were also evaluated. A single VF episode lasting more than 2 min was considered as sustained VF (sVF). Rats/hearts exhibiting sVF during ischemia were excluded from further evaluations. The duration of tachyarrhythmias was assessed as the duration of VT plus reversible VF.

**Echocardiography**

In the separate sets of SHR and SHR-CRP, evaluation of geometrical and functional parameters of the hearts was performed using echocardiographic system GE Vingmed System Seven with 14 MHz linear matrix probe [19]. Anesthesia was induced with 3% of isoflurane (Aerrane, Baxter SA) and then maintained at 2% during the ultrasound procedure. Rectal temperature was maintained within 36.5 and 37.5 °C by a heated table throughout measurements. Diastolic and systolic dimensions of LV were measured during echocardiographic evaluation including anterior and posterior wall thickness (AWTd, PWTd, AWTs, PWTs) and LV cavity diameter (LVDd, LVDs). From these dimensions, the following functional echocardiographic parameters were derived: fractional shortening (FS) = (LVDd-LVDs)/LVDd*100, ejection fraction (EF) = 100*(LVDd^3-LVDs^3)/LVDd^4, diastolic and systolic LV volumes (EDV, ESV) were calculated based on prolate spheroid geometry using the formula EDV = 0.001*(4*π/3)*k*LVDd^3/8 and ESV = 0.001*(4*π/3)*k*LVDs^3/8, where (k) is a ratio of long to short axis, stroke volume (SV) = EDV-ESV and cardiac output (CO) = SV*HR, where (HR) is heart rate.

After the measurement of baseline parameters, the functional reserve with serially infused dobutamine (1, 5, and 25 ng/g/min i.v. for 4 min each; Sigma-Aldrich, Czech Republic) was assessed [20].
**Blood pressure measurements**

In the separate sets of SHR and SHR-CRP, heart rate and mean arterial pressure were determined after three-day treatment by parasympathomimetic pyridostigmine (acetylcholinesterase inhibitor; 40 mg/kg/day in tap water; Sigma-Aldrich, Czech Republic) and in untreated conscious rats. For blood pressure measurement and drug application, polyethylene catheters (PE50 resp. PE10, Portex, Smith Medical International Ltd., UK) were inserted into the left carotid artery and jugular vein under isoflurane anaesthesia as mentioned above. Both catheters were filled with heparinized saline (500 I. U./ml, Heparin Léčiva, Zentiva, Czech Republic), tunnelled subcutaneously and exteriorized in the interscapular region. One day after the surgical procedures, the experiments were carried out in conscious rats kept in small transparent cages as described previously [21]. The signal from pressure transducer connected to bridge amplifier was digitalized with a computer based monitoring PowerLab system and recorded by LabChart software (ADInstruments Ltd, Australia). Heart rate was derived from arterial pressure signal as the reciprocal of pulse interval, which was computed as the interval between two consecutive systolic peaks. All animals were allowed to stabilize for a period of 30-min before measurements. To estimate the cardiac parasympathetic tone, methylatropine (a muscarinic AChR antagonist, which does not cross the blood-brain barrier; 2 mg/kg i.v.; Sigma-Aldrich, Czech Republic) were acutely administered to non-treated and pyridostigmine-treated SHR and SHR-CRP.

**Electrophysiological measurements and analysis**

ECG recordings were acquired in urethane (10 mg per 1 g of body weight; Sigma-Aldrich, Prague, Czech Republic) anesthetized non-infarcted rats. Needle 5-minute electrocardiograms (lead II) were recorded by Biopac System (Biopac Systems Inc., Santa Barbara, CA, USA) with a sampling rate of 1000 Hz. ECG intervals (RR, PR, QRS, QT) were measured by a blinded observer manually in 5 consecutive beats, averaged, and the mean values were used for further analyses and comparisons. Corrected QT intervals (QTc) were computed using Bazett’s formula normalized to average RR interval (QTc = QT/(RR/f)^1/2, f = 240 ms) [22].

Membrane potentials in the LV trabeculae were recorded as described previously [23]. Briefly, immediately after the animals were sacrificed by cervical dislocation, the hearts were excised and the papillary muscles with adjacent trabeculae were cut from the LV. The preparations were placed into a tissue bath perfused with oxygenated warm (37 °C) Tyrode solution (in mmol/L: NaCl 137, KCl 4.5, MgCl2 1, CaCl2 2, glucose 10, and HEPES 5; the pH was adjusted to 7.4 with NaOH; all chemicals were purchased from Sigma-Aldrich, Czech Republic) and stimulated at various frequencies (1, 2, 3, and 5 Hz; Pulsemaster Multi-Channel Stimulator A300, World Precision Instruments, Inc., FL, USA). Action potentials were measured using glass microelectrodes filled with 3M KCl (resistance >20 MΩ; Microelectrode Puller P-1000, Sutter Instruments, CA, USA). Action potential duration at 90% repolarization was measured offline in 10
consecutive cycles by in-house software made in MATLAB 2014b (MathWorks Inc., MA, USA). The results were averaged and used for statistical analyses.

**Optical mapping**

Intact animals of both strains were anesthetized by sodium pentobarbital as mentioned above. Hearts were rapidly excised and Langendorff-perfused in non-recirculating constant flow mode (10 mL/min per g) with Tyrode’s solution (in mmol/l: NaCl 145, KCl 5.9, CaCl$_2$ 1.1, MgCl$_2$ 1.2, glucose 11, HEPES 5; pH 7.4; 37°C) saturated with 100% O$_2$. After 10 min stabilization, the hearts were bolus stained with 200 μl of 0.125% di-4-ANNEPS (Invitrogen, ThermoFischer Scientific, Czech Republic) in DMSO (Sigma-Aldrich, Czech Republic) injected into a compliance chamber of the perfusion system as described earlier [24]. Additionally, reversible myosin II inhibitor blebbistatin (Sigma-Aldrich, Czech Republic) was added (50 μl in 0.4% DMSO) into the perfusion circuit to reduce undesirable heart motion. Recording was performed during sinus rhythm and during stimulation from the middle of the LV (300 ms cycle length, 2 ms duration, twice threshold) using the ULTIMA L camera fitted to BX51 WI epifluorescence microscope (Olympus, Japan) equipped with a150 W Xe arc lamp (Cairn, UK) as described [25]. Activation maps were constructed and conduction velocity (CV) parallel (longitudinal CV) and perpendicular (transversal CV) to the fiber direction were determined from the paced recordings. Heart rate was determined using BV_Analyzer software tools.

**Phospholipids fatty acids analysis**

In the separate sets of SHR and SHR-CRP, rats were killed by cervical dislocation; the heart was dissected on ice into the right ventricle (RV), LV and septum (S), each part was separately weighted, and LV samples were immediately frozen in liquid nitrogen and stored at −80°C until use for biochemical and metabolomic analyses.

Phospholipids from the LV were extracted according to Folch et al. [26] and lipid classes were separated by thin layer chromatography, fatty acids (FA) converted to methyl esters and analyzed by gas chromatography as described earlier [19] using gas chromatograph platform consisting of GC-FOCUS with automatic sampler Al 1310 (ThermoFisher Scientific, Italy). The FA profile of the phospholipids was used to calculate index reflecting the degree of unsaturation.

**Receptor density and enzyme activity**

Frozen LVs were combined and placed in the five-fold volume of ice-cold TMES buffer (20 mM TRIS, 3 mM MgCl$_2$, 1 mM EDTA, 250 mM sucrose, pH 7.4) containing protease and phosphatase inhibitors (cOMPLETE and PhosSTOP, Sigma-Aldrich, Czech Republic) and homogenized using Ultra Turrax (15 s, 24 000 rpm) on ice and then in a glass homogenizer with a motor-driven Teflon pestle at 1200 rpm for 2 min on ice. The homogenate was centrifuged (2100 rpm, 10 min, 4 °C), supernatant was collected and
pellet re-homogenized in TMES using glass-teflon homogenizer. After then, it was centrifuged again under the same conditions. Both supernatants were pooled and subsequently centrifuged (23 500 rpm, 30 min, 4 °C, Beckman Coulter Optima L.90K ultracentrifuge). Pellets (crude membrane fractions) were suspended in TME buffer (20 mM TRIS, 3 mM MgCl₂, 1 mM EDTA, pH 7.4) and washed. The resulting crude membranes were aliquoted and stored at -80 °C. Total number of β-adrenergic (β-AR) and muscarinic acetylcholine (AChR) receptors in crude membranes was measured using radioligand binding method in saturation arrangement with the non-selective β-AR antagonist [³H]CGP12177 and the AChR antagonist [³H]QNB, respectively, as described previously [27,28]. Adenyl cyclase (AC) activity was determined by measuring the conversion of [α-³²P]ATP to [³²P]cAMP [29].

Metabolomic and lipidomic analyses

Metabolomic and lipidomic profiling was conducted using a combined targeted and untargeted workflow for the lipidome, metabolome, and exposome analysis (LIMeX). For detailed description, see our recent study [30]. Briefly, LV samples (≈100 mg) were homogenized with 650 µL MeOH containing internal standards for 2 min using a grinder (MM400, Retsch, Germany), proteins precipitated at -80 °C for 30 min and separated from the supernatant by centrifugation (12 000 g, 10 min, 4 °C). Then, 600 µL of the supernatant was mixed with 3.38 mL of cold water with 0.01 mM HCl, the tubes were shaken for 1 min and samples purified using solid phase extraction columns (Strata-X 200 mg, Phenomenex, Torrance, CA, USA).

Lipid metabolites were extracted from tissue samples and plasma, respectively, using a biphasic solvent system of cold methanol, methyl tert-butyl ether, and water with some modifications. [31]. The bottom (polar) phase was collected and used for two LC-MS platforms. For hydrophilic interaction chromatography (HILIC) one aliquot was evaporated, resuspended in an acetonitrile/water (4:1, v/v) mixture followed by separation on an Acquity UPLC BEH Amide column (50x2.1 mm; 1.7 µm, Waters, The Netherlands) using gradient elution with acetonitrile/water (95:5, v/v) and water both solvents supplemented with 10 mM ammonium formate and 0.125% formic acid. The second aliquot was cleaned up using an acetonitrile/isopropanol mixture (1:1, v/v) and after evaporation, the dry extract was resuspended in 5% methanol with 0.2% formic acid followed by separation using reversed-phase liquid chromatography on an Acquity UPLC HSS T3 column (50x2.1 mm; 1.7 µm, Waters, The Netherlands) using gradient elution with water and methanol both solvents supplemented with formic acid (0.2% and 0.1%, respectively). Lipid metabolites were extracted using Strata-X 33u reverse phase extraction columns (200 mg/3 ml, Phenomenex, Torrance, CA, USA) according to manufacturer’s instructions.

In the separate sets of rats, animals were subjected to I/R in vivo as described above. One milliliter of blood was taken out from the carotid artery 15 min before coronary occlusion and 1 min before start of reperfusion. Blood was centrifuged and plasma aliquots were frozen in liquid nitrogen, and stored at -80 °C until use. The metabolites were extracted as described above. For LC-MS analysis, the systems consisted of
a Vanquish UHPLC System (Thermo Fisher Scientific, Bremen, Germany) coupled to a QExactive Plus mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) was used.

LC-MS was used to detect oxylipins [32,33]. For data processing, MS-DIAL software was used [34].

Statistics
The results are expressed as means ± SEM from the indicated number of experiments. Unpaired t test or Two-way ANOVA with subsequent Bonferroni test were used for comparison of differences in normally distributed variables between groups. Not normally distributed data (arrhythmias) are expressed as median ± interquartile range. Differences in the number of premature ventricular complexes between the groups were compared by the Mann-Whitney test. The incidence of tachycardia and fibrillation was examined by Fischer’s exact test. Differences were assumed statistically significant when \( P<0.05 \).
RESULTS

Ischemic and reperfusion ventricular arrhythmias and myocardial infarction in hearts in vivo

Detail analysis of I/R arrhythmias revealed a distinct proarrhythmic phenotype in SHR-CRP. The median of the total number of ischemic premature ventricular complexes (PVCs) was significantly increased to 3281 in SHR-CRP compared to 666 in SHR. Similarly, the median of tachyarrhythmias duration [ventricular tachycardia (VT) + reversible ventricular fibrillation (VF)] was significantly higher in SHR-CRP (336 s) than in SHR (32 s) (Fig. 1A, Table S1). One SHR-CRP died due to sustained VF (excluded from the study). Transgenic expression of CRP also tended to increase the incidence of VT and reversible VF. The median of the number of reperfusion PVCs was significantly higher in SHR-CRP than in SHR controls (Fig. 1B).

The area at risk (AR) normalized to LV did not differ between SHR-CRP (41.6 ± 4.0 %) and SHR (41.9 ± 1.9 %). Surprisingly, the infarct size (IS) normalized to AR was significantly lower in SHR-CRP (48.5 ± 5.1 %) compared with SHR (66.5 ± 4.9 %; Fig. 1C). Hence, the excess I/R arrhythmias were associated with the reduction of myocardial infarction in SHR-CRP hearts in vivo.

ECG, epicardial conduction velocities, and action potential duration analyses

We tried to explain a predisposition of SHR-CRP hearts to increased I/R arrhythmias using various electrophysiological approaches. ECG analysis did not show any significant differences in ECG intervals between the strains in non-infarcted hearts in vivo. However, the RR interval tended to decrease (P=0.059) in SHR-CRP (Fig. 2A). Optical mapping analysis of ex vivo perfused hearts revealed that the strains did not differ in HR, longitudinal and transversal epicardial conduction velocities, and the anisotropy ratio (longitudinal-to-transversal conduction velocity; Fig. 2B). Further analysis showed that transgenic expression of CRP did not affect the action potential duration at 90 % repolarization values measured in the LV trabeculae paced at various rates (Fig. 2C). In short, these experiments did not reveal any substantial electrophysiological abnormalities that would predispose SHR-CRP to a higher incidence of I/R arrhythmias.

Myocardial fatty acid composition and levels of eicosanoids

Given negative findings in the electrophysiological experiments, we focused on biochemical analysis of potential pro- and antiarrhythmic lipid mediators in the heart and plasma of non-infarcted rats. For these analyses, we prepared myocardial tissue and plasma samples from separate sets of animals; Table S2 summarizes the body and heart weights of SHR-CRP and SHR.

The myocardial proportion of n-3 polyunsaturated fatty acids (PUFAs) in phospholipids was significantly lower in SHR-CRP than in SHR, while n-6 PUFAs and both monounsaturated and saturated fatty acids (FAs) did not differ between the strains. The lower myocardial proportion of n-3 PUFA was reflected in the significant increase of n-6-to-n-3 PUFAs ratio by 13 % in SHR-CRP (Fig. 3A). A detailed FAs analysis revealed that this effect was due to a significantly lower concentration of the docosahexaenoic acid, a
predominant n-3 PUFA (Fig. 3A; Table S3). Further, myocardial abundances of antiarrhythmic epoxyeicosatrienoic acids (EETs) were decreased (for 14,15-EET significantly) in SHR-CRP compared to SHR. Moreover, the levels of EETs metabolites 11,12- and 14,15-dihydroxyeicosatrienoic acid increased by 93 and 117 %, respectively, but the differences did not reach statistical significance due to high variability (Fig. 3B,C). In plasma, we did not observe any significant changes at the levels of analyzed epoxides (Fig. 3B,C). Finally, the level of 6-keto prostaglandin F₁α (6-keto-PGF₁α), a non-enzymatic hydrolysis product of antiarrhythmic prostaglandin I₂ (PGI₂), significantly decreased by 44 % in plasma of SHR-CRP (Fig. 3D). On the other hand, the level of thromboxane B₂ (TXB₂), an inactive metabolite of proarrhythmic thromboxane A₂ (TXA₂) produced by platelets, increased by 78 % in plasma of SHR-CRP compared to SHR, but the difference did not reach statistical significance. In the LV myocardium, we did not observe any significant changes at the level of 6-keto-PGF₁α and TXB₂ (Fig. 3D). These findings suggest that an altered composition of myocardial and plasma lipids can be responsible, at least in part, for the proarrhythmic phenotype of SHR-CRP.

**Autonomic nervous system activity**

Autonomic nervous system activity exerts potent effects on the sensitivity of the heart to arrhythmias. Therefore, we determined the total number of muscarinic cholinergic and β-adrenergic binding sites in myocardial membrane preparations using saturation binding with the AChR agonist [³H]QNB and β-adrenergic receptor (β-AR) agonist [³H]CGP12177 (Fig. 4A,B). Analysis of saturation binding curves indicated that myocardial membranes prepared from SHR-CRP had a significantly lower number of AChRs than those from SHR. There was no difference in the binding affinity of these receptors (Fig. 4A). The levels of β-ARs were similar in both strains, but the dissociation constant (K₀) was significantly lower in SHR-CRP (Fig. 4B). Competition binding experiments with the β₂-AR selective antagonist (ICI 118.551) did not show any differences in the proportion of β₁- and β₂-ARs between the strains (Fig. 4B).

To evaluate the functional state of the myocardial AC signaling system, we investigated the activity of AC under various experimental conditions. The basal activity of AC and the AC activity stimulated by NaF (a G protein activator) or by forskolin (a direct activator of the catalytic subunit of AC) were comparable in myocardial preparations from both SHR-CRP and SHR. On the other hand, the AC activity stimulated by the β₁-AR agonist dobutamine and β₁/β₂-AR agonist isoprenaline was slightly but significantly lower in SHR-CRP than in SHR. The enzyme activity stimulated by the β₂-AR agonist salbutamol did not significantly differ between the groups (Fig. 4C).

The response of LV function to β-AR stimulation was examined by dobutamine stress echocardiography in a separate set of rats. Table S4 and Fig. 4D summarize echocardiographic parameters of LV. In untreated rats, the cardiac index did not differ between the strains (32.2 ± 1.8 vs. 32.2 ± 1.0 ml/min/100 g BW). Nevertheless, due to a slight LV dilation, transgenic expression of CRP significantly decreased the LV systolic function expressed as fractional shortening (FS) to 34.0 ± 0.5 % compared to 37.1
± 0.3 % in SHR (Fig. 4D, Table S4). Dobutamine increased maximal FS in both strains, but mild systolic dysfunction persisted in SHR-CRP (Fig. 4D). Altogether, these findings suggest that myocardial β-AR signaling did not substantially differ between the strains.

In additional experiments, MAP and HR were analyzed in conscious rats affected by pharmacological modulators of parasympathetic activity. In untreated SHR-CRP, MAP and HR were significantly higher (by 13.6 % and 10.7 %, respectively) compared to untreated SHR. Acute administration of methylatropine increased HR by 25.6 % in SHR and by 18.1 % in SHR-CRP (Fig. 4E). Three-day treatment with pyridostigmine did not affect SHR but significantly reduced MAP and tended to decrease HR in SHR-CRP to the values comparable with SHR. In both pyridostigmine-treated SHR and SHR-CRP, acute administration of methylatropine significantly increased HR compared with untreated groups; this effect was more pronounced in SHR (38.9 %) than in SHR-CRP (29.3 %) (Fig. 4E). Altogether, these data suggest that transgenic expression of human CRP in SHR resulted in autonomic nervous system imbalance due to suppressed parasympathetic activity.

**Relationship among the duration of tachyarrhythmias, blood pressure drop, and infarct size**

As the proarrhythmic phenotype of SHR-CRP hearts *in vivo* was associated with the reduction of myocardial infarction (Fig. 1), we supposed that these manifestations of acute I/R injury can be in a close relationship. To test this hypothesis, we performed a retrospective analysis of our previous data [17, 35] obtained in SHR under completely identical experimental conditions to expand the results of the present study.

Fig. 5A shows the relationship between the duration of ischemic ventricular tachyarrhythmias and IS normalized to AR in both strains. The majority of SHR (25 out of 29 rats) exhibited the duration of tachyarrhythmias lower than 200 s without any apparent influence on IS. However, four SHR (14 %) with the duration of tachyarrhythmias exceeding 200 s had lower IS than the rest of SHR. In SHR-CRP, 8 out of 10 rats had the duration of tachyarrhythmias longer than 200 s that was associated with an IS-limiting effect (Fig. 5A).

Severe ischemic arrhythmias can lead to episodes of blood pressure drop resulting in insufficient organ and tissue perfusion. Fig. 5B shows the individual values of time when MAP dropped below 40 mmHg due to ischemic ventricular tachyarrhythmias in SHR and SHR-CRP. Forty mmHg was chosen based on the previous animal studies that determined this value of MAP as close to the critical closing pressure for the whole circulation [36, 37]. During ischemia, MAP dropped below 40 mmHg in 3 out of 8 SHR and in 9 out of 10 SHR-CRP (Fig. 5B). In these animals (3 SHR and 9 SHR-CRP), the myocardial infarction was significantly reduced to 47.1 ± 3.5 % compared to 75.3 ± 3.5 % in those rats that did not exhibit sufficient MAP drop (Fig. 5B). The AR normalized to LV did not differ between the groups with or without MAP drop episodes (38.7 ± 3.7 % vs. 43.3 ± 2.6 %). In short, severe ischemic arrhythmias caused transient blood pressure drops associated with insufficient organ and tissue perfusion, which was accompanied by IS-limiting effect.
Plasma metabolomic analysis

We hypothesized that severe ischemic arrhythmias result in a release of hypoxic metabolites from underperfused organs and tissue to the blood that can reduce myocardial injury at reperfusion. To test this hypothesis, we performed metabolomic analysis of plasma before and at the end of myocardial ischemia in SHR and SHR-CRP (Table S5). Of all analyzed plasma metabolites collected before ischemia, 97.1 % did not differ between the strains. Acute myocardial ischemia significantly affected (predominantly elevated) 76.0 % of plasma metabolites in SHR-CRP and 36.5 % in SHR. In ischemic SHR-CRP, levels of most plasma metabolites (66 out of 79) were significantly different from those of ischemic SHR (Table S5). Specifically, myocardial ischemia increased plasma levels of compounds associated with tissue hypoxia and metabolites, reflecting the inhibition of oxidative metabolism in the organism. Indeed, lactate and pyruvate levels significantly increased in SHR-CRP (but not in SHR), suggesting organ and tissue hypoxia (Fig. 6A). Plasma levels of succinate, which is the Krebs cycle metabolite, significantly increased in both strains, but the effect was more pronounced in SHR-CRP than in SHR (Fig. 6A). The level of urea cycle substrate glutamate and its metabolites citrulline significantly increased at the end of ischemia in SHR-CRP only (Fig. 6B). The plasma level of orotate, the product of an alternative pathway of glutamate metabolism and substrate for pyrimidines synthesis, increased significantly, more in SHR-CRP than in SHR (Fig. 6B). Likewise, urate abundance, the product of purine degradation, significantly increased at the end of ischemia in SHR-CRP but not in SHR (Fig. 6C). Finally, the plasma level of acetoacetate [ketone bodies reacting with NADH and hydrogen ion and forming β-hydroxybutyrate (BHB)], significantly decreased in plasma at the end of ischemia in SHR-CRP only (Fig. 6D). Accordingly, the BHB level significantly increased, and the effect tended to be more pronounced in SHR-CRP than in SHR (Fig. 6D).

Remote ischemic perconditioning

Infarct size-limiting effect triggered by excess ischemic arrhythmias and mediated by a release of various hypoxic metabolites to the blood can imitate the cardioprotective action of RIPer [38,39]. To test this hypothesis, we analyzed myocardial ischemic tolerance in SHR subjected to RIPer (i.e., repeated occlusions of both hindlimbs during acute myocardial ischemia).

The incidence and severity of ischemic and reperfusion ventricular arrhythmias did not differ between perconditioned and naïve control SHR. RIPer only tended to reduce the incidence and number of severe ischemic arrhythmias (VT and VF) compared to controls (Fig. 7A,B and Table S1). The size of AR normalized to LV was similar in both groups (41.9 ± 1.9 % and 40.3 ± 5.8 %). In perconditioned SHR, the IS normalized to AR was significantly smaller than in controls (46.1 ± 3.9 % vs. 66.5 ± 4.9 %; Fig. 7C). Thus, RIPer resulted in an IS-limiting effect in SHR (without significant changes in I/R arrhythmias), which was comparable with myocardial infarction observed in SHR-CRP.
Ischemic and reperfusion ventricular arrhythmias and myocardial infarction in hearts ex vivo

If our hypothesis of the IS-limiting effect caused by metabolites released to plasma due to hypoxia (as a consequence of severe ischemic tachyarrhythmias) is correct, the isolated perfused hearts of SHR-CRP subjected to ischemia will not show smaller IS than the hearts of SHR.

Fig. 8A,B and Table S1 demonstrate ischemic and reperfusion arrhythmias in SHR and SHR-CRP hearts ex vivo. In SHR, the incidence of VT reached 100 %, and the incidence of VF reached 12.5 %. One of SHR-CRP hearts had sustained VF (excluded from the study) and the incidences of VT and reversible VF were similar to those of SHR (100 and 18.2 %, respectively; Fig. 8A). The incidence and severity of both ischemic and reperfusion arrhythmias did not differ between the strains in any parameter (Fig. 8A,B and Table S1).

In SHR-CRP and SHR, the size of AR normalized to LV was comparable (38.5 ± 2.8 and 43.8 ± 2.8 %, respectively; Fig. 8C). The IS normalized to AR reached 50.0 ± 4.3 % in SHR-CRP and did not significantly differ from that in SHR (43.8 ± 2.5 %; Fig. 8C). Finally, we did not observe any relationship between the duration of ventricular tachyarrhythmias and IS normalized to AR in either strain subjected to I/R ex vivo. The duration of tachyarrhythmias exceeding 200 s (3 out of 18 hearts) did not reduce myocardial infarction (Fig. 8D). Therefore, ventricular tachyarrhythmias per se were unable to trigger an IS-limiting effect without the response of other organs and tissue.
DISCUSSION

In this study, transgenic expression of human CRP predisposed SHR-CRP to repeated and prolonged ischemic ventricular tachyarrhythmias. The proarrhythmic phenotype was associated with an altered composition of myocardial and plasma lipids and suppressed parasympathetic activity. Unexpectedly, the excessive ischemic arrhythmias in SHR-CRP hearts in vivo were connected with a significant reduction of myocardial infarction. It seems unlikely that the IS-limiting effect in SHR-CRP is a direct consequence of transgenic expression of human CRP. Our data indicate that insufficient organ and tissue perfusion caused by severe ischemic tachyarrhythmias triggers the release of various hypoxic metabolites in blood, which reduces myocardial injury at reperfusion.

Proarrhythmic phenotype in SHR-CRP

As electrophysiological experiments did not reveal any abnormalities related to high ischemic and reperfusion arrhythmogenesis in SHR-CRP, we focused on biochemical analyses of selected myocardial and plasma lipids that could be responsible for the proarrhythmic phenotype. In experimental animal models, a long-lasting intake of food enriched with n-3 PUFAs decreases the myocardial phospholipid n-6/n-3 PUFA ratio and diminishes the incidence and severity of ventricular arrhythmias during I/R [40-43]. In patients, higher consumption of n-3 PUFAs reduces the incidence of coronary heart disease and sudden cardiac death [44,45]; the inverse relationship between plasma CRP and n-3 PUFAs levels was reported [46-48]. Our study expands previous research by demonstrating that the excessive I/R arrhythmias in SHR with transgenic overexpression of human CRP is associated with lower content of n-3 PUFAs in myocardial phospholipids.

Myocardial or plasma eicosanoids, biologically active metabolites of arachidonic acid (AA), can modify heart sensitivity to ventricular arrhythmias. It has been shown that increased levels of EETs, epoxides produced from AA, protect hearts against electrical instability in mice subjected to pressure overload or I/R [49-52]. Similarly, pharmacological interventions that increase availability of EETs reduce the incidence of life-threatening ventricular fibrillation in hypertensive Ren-2 transgenic rats subjected to I/R [53]. In our SHR-CRP transgenic rats, myocardial, but not plasma, levels of antiarrhythmic EETs decreased, while their less biologically active metabolites dihydroxyeicosatrienoic acids increased compared to SHR. Thus, we speculate that lower myocardial levels of EETs could contribute to the increased ischemic arrhythmogenesis in SHR-CRP. Concerning EETs in plasma, their role in cardiac sensitivity to acute I/R arrhythmias seems unlikely. Accordingly, no association of plasma EETs with CRP levels in patients with established coronary artery disease was observed [54].

TxA$_2$ and PGI$_2$ represent important pro- and antiarrhythmic lipids derived from prostaglandin H$_2$ [55,56]. Platelets-synthetized TxA$_2$ has proarrhythmic action and pharmacological inhibition of its synthesis protects the heart against I/R-induced arrhythmias [57,58]. In contrast to TxA$_2$, acute exogenous administration of PGI$_2$ reduces the incidence of I/R arrhythmias [59-61]. In this study, lower plasma levels of
6-keto-PGF₁α (a non-enzymatic hydrolytic product of antiarrhythmic PGI₂) and higher TxB₂ levels (a stable and inactive metabolite of proarrhythmic TxA₂) were detected in SHR-CRP with the excessive ischemic arrhythmias. It is in line with the above mentioned reports and it also corresponds with studies determining TxA₂ and PGI₂ as important metabolites predisposing to ventricular arrhythmias in other experimental settings [62-66]. Concerning the relationship between CRP, TxA₂, and PGI₂, Grad et al. [67] showed that transgenic overexpression of human CRP suppressed PGI₂ synthase and augmented TxA₂ activity through an increase in the expression of TxA₂ receptor in mice. An increased plasma level of CRP was also associated with an elevated plasma level of TxB₂ in smokers with chronic coronary artery disease [68] and with increased urinary TxB₂ excretion in SHR [69]. Therefore, these findings suggest that CRP can modulate TxA₂ and PGI₂ activity and their biosynthesis or degradation and that could modify risk of excessive ischemic arrhythmias.

High incidence of ischemic and reperfusion ventricular arrhythmias in SHR-CRP was also associated with an autonomic nervous system imbalance, as suggested by a lower number of AChRs in LV and by decreased HR and MAP after parasympathomimetic treatment. Indeed, enhanced sympathetic or insufficient parasympathetic nervous activity can substantially contribute to the occurrence of severe ventricular arrhythmias under I/R [70] and during heart failure progression [71]. It has been shown that systemic inflammation (measured as a plasma level of CRP) is associated with decreased vagal heart rate control in patients with coronary artery disease [72]. In large animal models, vagal nerve stimulation reduced an elevated plasma CRP level and attenuated heart failure progression [73], suppressed acute ischemic and reperfusion ventricular arrhythmias [74], and diminished an increased post-MI ventricular arrhythmias inducibility [75]. Thus, autonomic nervous system imbalance and inflammation are mutually related [76]. In line with our present findings, it has been shown that increased TxA₂/PGI₂ plasma ratio can limit vagal tone [77] while n-3 PUFAs diet can increase vagal tone [78]. Taken together, our data suggest that altered composition of myocardial and plasma lipids as well as autonomic nervous system imbalance can potentially contribute to the excessive ischemic arrhythmogenesis in SHR-CRP hearts in vivo. However, the fact that I/R arrhythmias did not differ between the strains when assessed in hearts ex vivo argues for a dominant role of extracardiac factors. Further targeted experiments are needed to unravel the mechanism of increased arrhythmogenesis in SHR-CRP.

**Infarct size-limitation in SHR-CRP**

In contrast to the excessive ischemic arrhythmias, myocardial infarction under in vivo conditions was significantly smaller in SHR-CRP compared to SHR. This result is in contradiction to generally considered harmful role of CRP in ischemic heart diseases [1-4]. To the best of our knowledge, no experimental or clinical data suggesting CRP-associated cardioprotective action are available. Therefore, we tried to explain this unexpected finding by including a retrospective analysis of our previous data on ischemic arrhythmias and infarct size in SHR subjected to I/R in vivo. Like SHR-CRP, those few SHR that showed an extreme total
duration of ischemic tachyarrhythmias were also protected against myocardial infarction. Therefore, we hypothesized that insufficient organ and tissue perfusion caused by repeated and prolonged ischemic tachyarrhythmias led to hypoxia, resulting in a release of cardioprotective metabolites in blood, which could reduce myocardial injury at reperfusion. The following observations support this view. First, we show that the IS-limiting effect manifested only in those animals that exhibited episodes of repeated and prolonged ventricular tachyarrhythmias resulting in substantial blood pressure drops, which temporally compromised blood supply to tissue. Second, RPer induced by repeated hindlimb occlusions during myocardial ischemia reduced the IS in SHR (without significant changes in I/R arrhythmias) to a value comparable to IS observed in naïve SHR-CRP. Third, the I/R insult in ex vivo hearts did not reveal any differences in IS between the strains, indicating that the body hypoxic response was required for the manifestation of improved myocardial ischemic tolerance.

In line with this view, our metabolomic analyses showed that acute myocardial infarction significantly affected plasma levels of many metabolites at the end of ischemia in SHR-CRP. The increased levels of some of these metabolites reflect not only organ and tissue hypoxia, but they were also identified as potent cardioprotective molecules in previous studies. For example, pyruvate was revealed as a powerful protectant against acute myocardial I/R injury [79]. It has been suggested that the accumulation of succinate may improve ischemic energetics. Moreover, extracellular succinate may serve as a signaling molecule at reperfusion [80], as it promotes hypoxia-inducible factor-1α (HIF-1α) activation by inhibition of HIF-1α-degrading enzymes prolyl hydroxylases [81], thereby mediating cardioprotection [82,83]. Further, high plasma levels of glutamate or orotate at the start of reperfusion can also provide cardioprotection [84-86]. It has also been shown that acute elevation of urate mediates antioxidant defense against peroxynitrite- and tyrosine nitration-induced damage and protects hearts against I/R injury [87,88]. Last but not least, exogenously administered ketone body BHB before reperfusion reduced I/R injury in mice and rats [89,90]. Similarly, 3-days fasting associated with BHB level elevation limited the extent of acute myocardial infarction in rats [91]. Further studies are needed to reveal individual contributions of these metabolites to cardioprotective signaling elicited by excess ischemic arrhythmias.

The fact that our experiments were performed on rats can be considered a certain limitation for the potential translation of the results. Indeed, there are major species differences, in particular between small rodents, large animals and human, in ventricular ion channel function, susceptibility to arrhythmias and their lethality [92,93]. For example, while VF episodes induced by coronary occlusion usually self-terminate in rats, myocardial ischemia in pigs and humans often leads to sustained VF, which without external defibrillation results in death [93,94]. Malignant ischemic ventricular tachyarrhythmias are a common cause of sudden cardiac death in humans [95]. However, it cannot be excluded that our finding also applies to other species and may have potential clinical relevance in patients with acute MI accompanied by excess ventricular arrhythmias. Elucidation of the underlying mechanism may be beneficial in the search for novel protective strategies against acute myocardial I/R injury.
Conclusion

Our experiments demonstrate a new form of myocardial protection against infarction that is likely mediated by protective substances released from body organs and tissues made hypoxic due to exaggerated ischemic tachyarrhythmias. This form of cardioprotection is initiated by the heart itself without any external intervention (i.e., "self-conditioning") such as a targeted short occlusion of another organ as was demonstrated for various forms of remote conditioning. It seems unlikely that a decline in coronary perfusion and myocardial hypoxia play a major role in the protective mechanism because we did not observe any reduction of infarct size in isolated SHR-CRP hearts perfused under ex vivo conditions.

This mechanism of infarct size reduction is most likely not limited to SHR-CRP strain, as the same effect was observed in some progenitor SHR. Moreover, in our ongoing study, excess ischemic tachyarrhythmias were associated with the reduced infarct size also in transgenic rats with ANG II-dependent malignant hypertension (unpublished). It seems, therefore, that the infarct size limitation triggered by repeated and prolonged ventricular tachyarrhythmias during ischemia may represent a more general phenomenon. Based on our present data, we strongly encourage performing an analysis of arrhythmias occurring during I/R experiment in vivo in addition to IS determination. It can help to avoid misleading interpretations of cardiac ischemic tolerance, in particular in transgenic animal models with various comorbidities.
Clinical Perspective

- The role of human C reactive protein (CRP) in acute myocardial ischemia/reperfusion injury was studied in spontaneously hypertensive rats (SHR) with transgenic expression of CRP (SHR-CRP) and SHR controls.
- SHR-CRP exhibited exaggerated ischemic tachyarrhythmias that reduced myocardial infarct size due to a drop of perfusion pressure and subsequent release of protective substances from hypoxic organs and tissues.
- This new form of myocardial protection, i.e. the infarct size limitation triggered by the heart itself without any external intervention, may represent a more general phenomenon. Elucidation of its mechanism may be beneficial in the search for novel protective strategies against acute myocardial ischemia/reperfusion injury.
Data Availability
The authors confirm that the data supporting the findings of the present study are available within the article (and/or) its supplementary materials.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

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Author contributions
Study design: JNe and FK; acquisition and analysis of data: JNe, PA, VO, FP, LH, JŠ, MBeh, MBen, JH, MV, DJ, JŠ and EM; interpretation of data: JNe, VO, FP, MŠ, JNo, BO, MP and FK; drafting of manuscript: JNe, JNo, MŠ and FK; final revision of manuscript: JNe and FK.

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Abbreviations and Acronyms: AA, arachidonic acid; AC, adenylyl cyclase; AChR, acetylcholine receptor; AR, area at risk; β-AR, β-adrenergic receptor; BHB, β-hydroxybutyrate, CRP, C-reactive protein; EET, epoxyeicosatrienoic acid; FAs, fatty acids; HIF-1α, hypoxia-inducible factor-1α; IS, infarct size; I/R, ischemia/reperfusion; 6-keto-PGF1α, 6-keto prostaglandin F1α; PGI2, prostaglandin I2; PUFAs, polyunsaturated fatty acids; PVCs, premature ventricular complexes; RIPer, remote ischemic perconditioning; SHR, spontaneously hypertensive rats; SHR-CRP, spontaneously hypertensive rats expressing human CRP transgene; TXA2, thromboxane A2; TXB2, thromboxane B2; VF, ventricular fibrillation; VT, ventricular tachycardia.
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FIGURE LEGENDS

Fig. 1 Ischemic and reperfusion ventricular arrhythmias and myocardial infarction in hearts in vivo
The incidence of ventricular tachycardia (VT) and fibrillation (VF), the total number of premature ventricular complexes (PVCs), and the duration of tachyarrhythmias (VT + reversible VF) (A); the number of reperfusion PVCs (B); the relative size of the area at risk and the infarct size in hearts in vivo with representative images of staining (C) in control spontaneously hypertensive rats (SHR) and transgenic SHR-CRP. Values are shown as median with interquartile range (number of PVCs and duration of tachyarrhythmias) and as mean ± SEM of 8-10 hearts in each group. *P<0.05 and **P<0.001 vs. SHR by Mann-Whitney test (number of PVCs and duration of tachyarrhythmias), by Fischer’s exact test (incidence of VT and VF), and by unpaired t-test (relative size of the area at risk and infarct size).

Fig. 2 ECG, epicardial conduction velocity, and action potential duration analyses
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Fig. 3 Myocardial fatty acid composition and levels of eicosanoids
The total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), n-3 and n-6 polyunsaturated fatty acids (PUFA), n-6/n-3 PUFA ratio, and docosahexaenoic fatty acid (DHA) proportion in the total phospholipids in the left ventricle (A). Myocardial and plasma levels of 14,15-epoxyeicosatrienoic (EET), 14,15-dihydroxyeicosatetraenoic acids (DHET) (B), 11,12-EET and 11,12-DHET (C), and 6-keto prostaglandin F$_{1α}$ (6-keto PGF$_{1α}$) and thromboxane B$_2$ (TxB$_2$) (D) in the control spontaneously hypertensive rats (SHR) and transgenic SHR-CRP. Values are mean ± SEM of 6-8 hearts in each group. *P<0.05 vs. SHR by unpaired t-test.

Fig. 4 Autonomic nervous system activity
Saturation binding curves of muscarinic cholinergic (A) and β-adrenergic receptors (β-ARs; B) constructed by measurement of $[^3]$H]QNB and $[^3]$H]CGP 12177, respectively, binding to crude myocardial membranes using increasing concentrations of the radioligand. The distribution of β-ARs subtypes in myocardial preparations was assessed by competitive displacement of $[^3]$H]CGP 12177 binding by the β$_2$-AR selective antagonist ICI 118.551 (B). Adenylyl cyclase activity under basal conditions and after stimulation by salbutamol, dobutamine, isoproterenol, NaF, and forskolin in left ventricle of control spontaneously hypertensive...
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Relationship between infarct size (normalized to the area at risk) and the total duration of ischemic ventricular tachyarrhythmias in spontaneously hypertensive rats (SHR; n=29) and transgenic SHR-CRP (n=10). Data are from Neckar et al. [17,35] (red circles) and the present study (SHR – black circles; SHR-CRP – open circles) obtained under completely identical experimental conditions (A). The individual values of time when mean arterial pressure (MAP) dropped below 40 mmHg, and the infarct size in hearts of SHR and transgenic SHR-CRP with or without MAP drops below 40 mmHg (B). Values are shown as mean ± SEM of 8-10 hearts in each group. ***P<0.001 vs. SHR by unpaired t-test.

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Plasma levels of selected metabolites associated with tissue hypoxia and oxidative metabolism (A), urea cycle (B), catabolism of purine nucleotides (C) and formation of ketone bodies (D) before (Pre-Isch) and at the end (End-Isch) of 20-min ischemia in the control spontaneously hypertensive rats (SHR) and transgenic SHR-CRP. Values are mean ± SEM of 7-8 hearts in each group; *P<0.05, **P<0.01, and ***P<0.001 vs. SHR or Pre-Isch group by Two-way ANOVA with Bonferroni’s test.

Fig. 7 Ischemic and reperfusion ventricular arrhythmias and myocardial infarction in SHR hearts in vivo subjected to remote ischemic perconditioning
The incidence of ventricular tachycardia (VT) and fibrillation (VF), the total number of premature ventricular complexes (PVCs) and duration of tachyarrhythmias (VT + reversible VF) (A); the number of reperfusion PVCs (B); the relative size of the area at risk and the infarct size (C) in hearts of control spontaneously hypertensive rats (SHR) in vivo and SHR subjected to remote ischemic perconditioning (RIPer). Data for SHR group were copied from Figure 1. Values are shown as median with interquartile range (number of PVCs and duration of tachyarrhythmias) and as mean ± SEM of 7-8 hearts in each group. Data were analyzed by Mann-Whitney test (number of PVCs and duration of tachyarrhythmias), by Fischer’s exact test (incidence of VT and VF) and by unpaired t-test (relative size of the area at risk and infarct size).
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