Alternative cardioprotective approaches and their effects on myocardial peroxynitrite, RISK and SAFE pathways

Summary of PhD thesis

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List of publications

1. List of full papers directly related to the subject of the thesis

I. **Pipicz M.**; Kocsis G.F.; Sarvary-Arantes L.; Bencsik P.; Varga Z.V.; Ferdinandy P.; Csont T. Low-dose endotoxin induces late preconditioning, increases peroxynitrite formation, and activates STAT3 in the rat heart. *Molecules* 2017, 22. [IF: 2.861]

II. **Pipicz M.**; Varga Z.V.; Kupai K.; Gaspar R.; Kocsis G.F.; Csonka C.; Csont T. Rapid ventricular pacing-induced postconditioning attenuates reperfusion injury: Effects on peroxynitrite, RISK and SAFE pathways. *British Journal of Pharmacology* 2015, 172, 3472-3483. [IF: 5.259]

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2. List of other full papers

I. Kocsis G.F.; Sarkozy M.; Bencsik P.; **Pipicz M.**; Varga Z.V.; Paloczi J.; Csonka C.; Ferdinandy P.; Csont T. Preconditioning protects the heart in a prolonged uremic condition. *American Journal of Physiology. Heart and Circulatory Physiology* 2012, 303, H1229-1236. [IF: 3.708]

II. Varga Z.V.; Zvara A.; Farago N.; Kocsis G.F.; **Pipicz M.**; Gaspar R.; Bencsik P.; Gorbe A.; Csonka C.; Puskas L.G.; Thum T.; Csont T.; Ferdinandy P. MicroRNAs associated with ischemia-reperfusion injury and cardioprotection by ischemic pre- and postconditioning: ProtectomiRs. *American Journal of Physiology. Heart and Circulatory Physiology* 2014, 307, H216-227. [IF: 3.838]

III. Csont T.; Murlasits Z.; Menesi D.; Kelemen J.Z.; Bencsik P.; **Pipicz M.**; Fekete V.; Zvara A.; Puskas L.G.; Ferdinandy P. Tissue-specific gene expression in rat hearts and aortas in a model of vascular nitrate tolerance. *Journal of Cardiovascular Pharmacology* 2015, 65, 485-493. [IF: 2.462]

IV. Sarkozy M.; Szucs G.; **Pipicz M.**; Zvara A.; Eder K.; Fekete V.; Szucs C.; Barkanyi J.; Csonka C.; Puskas L.G.; Konya C.; Ferdinandy P.; Csont T. The effect of a preparation of minerals, vitamins and trace elements on the cardiac gene expression pattern in male diabetic rats. *Cardiovascular Diabetology* 2015, 14, 85. [IF: 4.534]

V. Schreckenberg R.; Rebelo M.; Deten A.; Weber M.; Rohrbach S.; **Pipicz M.**; Csonka C.; Ferdinandy P.; Schulz R.; Schluter K.D. Specific mechanisms underlying right heart failure: The missing upregulation of superoxide dismutase-2 and its decisive role in antioxidative defense. *Antioxidants & Redox Signaling* 2015, 23, 1220-1232. [IF: 7.093]

VI. Csonka C.; Sarkozy M.; **Pipicz M.**; Dux L.; Csont T. Modulation of hypercholesterolemia-induced oxidative/nitrative stress in the heart. *Oxidative Medicine and Cellular Longevity* 2016, 2016, 3863726. [IF: 4.593]

VII. Gaspar R.; **Pipicz M.**; Hawchar F.; Kovacs D.; Djirackor L.; Gorbe A.; Varga Z.V.; Kiricsi M.; Petrovski G.; Gacser A.; Csonka C.; Csont T. The cytoprotective effect of biglycan core protein involves Toll-like receptor 4 signaling in cardiomyocytes. *Journal of Molecular and Cellular Cardiology* 2016, 99, 138-150. [IF: 5.680]

VIII. Sarkozy M.; Szucs G.; Fekete V.; **Pipicz M.**; Eder K.; Gaspar R.; Soja A.; Pipis J.; Ferdinandy P.; Csonka C.; Csont T. Transcriptomic alterations in the heart of non-obese type 2 diabetic Goto-Kakizaki rats. *Cardiovascular Diabetology* 2016, 15, 110. [IF: 4.752]

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1. Introduction

1.1. Ischemic heart disease: an epidemiological burden

Ischemic heart diseases are the leading cause of death worldwide and characterized by restriction in blood supply to the heart. Regarding the number of disease-specific mortality, acute myocardial infarction is one of the major emergency manifestation of ischemic heart diseases, and occurs when a coronary artery is suddenly occluded, thereby restricting blood supply to the myocardium.

1.2. Ischemia/reperfusion injury: concept of the phenomenon

As a result of hypoxia and deprivation of nutrients, prolonged myocardial ischemia leads to time-dependent cell death (i.e. ischemic injury). The procedure that allows rapid return of blood flow to the ischemic myocardium is termed reperfusion therapy, which decreases infarct development and mortality. However, early reperfusion period itself is accompanied by deleterious events such as life-threatening arrhythmias, no-reflow phenomenon, myocardial stunning and additional cell death as well, which is called reperfusion injury.

1.3. Classic cardioprotective methods: ischemic conditionings

Ischemic pre- and postconditioning are strategies for protecting the heart against the detrimental effects of ischemia/reperfusion injury, by means of application of brief non-harmful ischemia/reperfusion cycles before or after a prolonged lethal ischemia, respectively, to elicit endogenous cardioprotective mechanisms. The cardioprotective effect of preconditioning is biphasic with an early phase (lasts for hours) and a late phase (starts 12 h after preconditioning stimuli and lasts for ~72 h).

1.4. Mechanism of ischemic conditionings

Both ischemic conditioning approaches may partly share common molecular mechanisms: promote formation of trigger molecules which act on receptor dependent or independent pathways to activate a final end-effector, thereby exerting cardioprotection.

Peroxynitrite has emerged as non-conventional trigger of ischemic conditionings and arises from the non-enzymatic reaction of superoxide ($O_2^{\bullet-}$) with nitric oxide ($NO^{\bullet}$). Xanthine oxidoreductase (XOR) and nitric-oxide synthases (NOS) are the primary sources of $O_2^{\bullet-}$ and $NO^{\bullet}$, respectively. Peroxynitrite is suggested to activate pro-survival signaling pathways.

Reperfusion injury salvage kinase- (RISK) and survivor activating factor enhancement- (SAFE) pathways are recruited during reperfusion, and elicited by ischemic conditionings for cell survival. RISK represents the extra-cellular signal-regulated kinase 1 and 2 (ERK1/2) and

protein kinase B (Akt), while signal transducer and activator of transcription 3 (STAT3) is the key member of SAFE. Both pathways promote pro-survival cellular processes.

1.5. Alternative approaches: other ways to confer cardioprotection

Ischemic pre- and postconditioning seem to be effective cardioprotective approaches; nevertheless, there are many limitations and confounding factors (e.g. failure to achieve complete reperfusion during application of brief ischemia/reperfusion cycles, the algorithm of maneuver, presence of comorbidities) which indicate the necessity of alternative methods of classic ischemic conditionings. For instance, both conditioning methods can be elicited by a wide variety of pharmacological (e.g. endotoxin, antioxidants, cyclosporine) and non-pharmacological (e.g. rapid ventricular pacing, heat stress, exercise) stimuli as well to confer cardioprotection.

A well-documented cardioprotective drug is the gram-negative bacterial lipopolysaccharide (LPS) endotoxin. Specifically, administration of low-dose endotoxin 24 h before a test ischemia/reperfusion has been shown to improve post-ischemic cardiac functional recovery, thereby exerting pharmacological late preconditioning. The exact mechanism of endotoxin-induced late preconditioning is not entirely clear. Increasing evidence suggests that enhanced formation of cardiac peroxynitrite plays a role in late phase of ischemia-induced delayed preconditioning; however, data is still lacking regarding the delayed effect of low-dose LPS on peroxynitrite formation in the heart. Cardioprotective signaling pathways are barely investigated in late preconditioning elicited by LPS. The activation of Akt was shown to play a role in LPS-induced late preconditioning; nevertheless, potential implication of ERK1/2, another RISK kinases, and STAT3, the key member of SAFE, has not yet been tested.

It is well established that beside the pharmacological approaches many other ways exist to recruit endogenous adaptive mechanisms. For instance, heart rate is suggested to play a role in the development of ischemia/reperfusion injury, and applying short periods of rapid ventricular pacing before an index ischemia has anti-ischemic effects (pacing-induced preconditioning). Nevertheless, it is not known whether ventricular tachyarrhythmias (like ventricular tachycardia (VT) and/or ventricular fibrillation (VF)), developed spontaneously in response to the reperfusion, can influence infarct size, and whether rapid ventricular pacing applied after an index ischemia, as a possible novel alternative postconditioning strategy, attenuates reperfusion injury. Moreover, the effects of short periods of rapid ventricular pacing on myocardial peroxynitrite, and possible downstream signaling targets are not known.

2. Goals of the thesis

Developing and testing of alternative approaches is indispensable to elucidate and understand cardioprotection. In order to improve our knowledge, in this thesis we focused on two distinct alternative conditioning methods of ischemic pre- and postconditioning, i.e. LPS-induced late preconditioning and rapid ventricular pacing-induced postconditioning. With the purpose of fulfilling the above mentioned gaps in the current knowledge, the two proposed alternative methods were tested and the following specific questions were addressed:

1. Has the low-dose cardioprotective LPS any delayed effect on myocardial peroxynitrite formation in endotoxin-induced late preconditioning?
2. How does the low-dose LPS treatment affect the cardiac RISK and SAFE pathways in the late phase of endotoxin-induced preconditioning?
3. Is there an association between the duration of reperfusion-induced ventricular tachyarrhythmias (VT, VF, or VT+VF) and infarct size?
4. Could the short periods of rapid ventricular pacing performed at the early phase of reperfusion attenuate reperfusion injury and induce postconditioning?
5. Is peroxynitrite potentially involved in the rapid ventricular pacing-induced postconditioning?
6. What is the effect of rapid ventricular pacing-induced postconditioning on the possible myocardial downstream targets, RISK and SAFE pathways?

3. Materials and methods

Regarding both alternative conditioning approaches, several experimental designs were set-up on isolated rat heart preparations to address our aims.

3.1. Experimental designs

3.1.1. LPS-induced late preconditioning

Male Wistar rats were treated intraperitoneally with saline or low-dose (0.5 mg/kg) LPS from *Salmonella enterica* serotype *typhimurium* (n = 6–7 in both groups). Twenty four hours after LPS treatment, hearts were isolated and perfused according to Langendorff for 5 min. Then the perfusion system was switched to working mode according to Neely with recirculating buffer. Hearts were subjected to 10 min equilibration period followed by 30 min normothermic global ischemia and 20 min reperfusion. Before and after the ischemia cardiac functional parameters and lactate dehydrogenase (LDH) activity in coronary effluents were measured. In separate experiments, hearts were harvested at the end of a 5-min Langendorff perfusion for biochemical analyses (n = 5–12 in both groups). After removing atria, ventricles were used freshly or were rapidly freeze-clamped, powdered with a pestle and mortar in liquid nitrogen, and stored in cryovials at -80 °C until further analysis of myocardial peroxynitrite, RISK and SAFE pathways.

3.1.2. Rapid ventricular pacing-induced postconditioning

To address the question whether there is an association between the duration of reperfusion-induced ventricular tachyarrhythmias and infarct size, a meta-analysis was performed. Electrocardiograms and infarct size data were analyzed from our six previous studies done in our laboratory on isolated rat hearts subjected to 30 min regional ischemia and 120 min reperfusion. Reperfusion-induced arrhythmias were analyzed in the first 10 min of reperfusion, and evaluations were done based on total duration of VT, VF, or VT+VF.

In order to examine whether rapid ventricular pacing applied at the onset of reperfusion induces cardioprotection, the following perfusion protocol was performed. The ischemia/reperfusion control group was subjected to 15 min equilibration period, 30 min regional index ischemia and 120 min reperfusion. Ischemic postconditioning was induced by six consecutive cycles of 10 s reperfusion and 10 s no-flow global ischemia at the onset of reperfusion. In the rapid ventricular pacing group the spontaneous rhythm of hearts was replaced by 10-s pacing period (600 bpm; 10 Hz) in 6 alternating cycles during the first 2 min of reperfusion. Post-ishemic LDH release (n = 5 in each group), infarct size (n = 12 in each

group) and reperfusion-induced tachyarrhythmias (n = 11–14 in each group) were assessed. In separate experiments using similar protocol, at the end of a 7-min reperfusion ischemic zone of the left ventricles was used for analyzing myocardial peroxynitrite, RISK and SAFE pathways (n = 5 in each group), as described above.

3.2. Isolated heart preparation

Male Wistar rats (250 – 400 g) were used in our experiments. Rats were anesthetized with diethyl ether, an anesthetic not known to interfere with cardioprotection. Inhalation anesthesia was induced in a glass desiccator containing cellulose wadding soaked in diethyl ether. During isolation of the heart, rats were removed from the chamber and a beaker containing wadding soaked in ether was held near the muzzle of rats in order to maintain anesthesia. Rats were given 500 U/kg heparin intravenously. Hearts were then isolated and perfused according to Langendorff at 37 ºC with Krebs-Henseleit buffer containing NaCl 118 mM, $NaHCO_3$ 25 mM, KCl 4.3 mM, $CaCl_2$ 2.4 or 1.4 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, glucose 11 mM, gassed with 95% O_2 and 5% CO_2. Hydrostatic perfusion pressure was kept constant at 100 cmH_2O (9.8 kPa) throughout the experiments. In recirculating working mode according to Neely, preload and afterload were kept constant at 1.7 kPa and 9.8 kPa, respectively throughout the experiments. Coronary flow was measured by collecting coronary effluent for a period of time and was expressed as mL/min.

No-flow global ischemia was performed by turning off the perfusion cannula. To induce regional index ischemia, a 3-0 silk suture was placed around the left anterior descending coronary artery close to its origin and the snare was tightened by applying a 100 g hanging weight. The presence of ischemia was verified by monitoring coronary flow.

Rapid ventricular pacing (600 bpm; 10 Hz) was performed by an electric stimulator (Experimetria, Budapest, Hungary) with double threshold square, 1 V, 1 mA and 5-ms impulses conducted by electrodes attached directly to the surface of the right ventricle close to the apex and to the aortic cannula.

3.3. Lactate dehydrogenase assay

In order to assess myocardial injury, the activity of LDH enzyme from coronary effluents (collected for 5 min) was measured using a LDH-P kit. The enzyme activity (U/mL) measured in an effluent was multiplied with the corresponding coronary flow (mL/min) to give LDH release expressed as U/min.

3.4. Infarct size measurement

At the end of reperfusion the left anterior descending coronary artery was reoccluded and area at risk was determined by Evans blue. Then heart slices were incubated in 2,3,4-triphenyl-tetrazolium-chloride solution to visualize viabale tissues. Finally, infarct size was determined by planimetry and normalized to area at risk.

3.5. Evaluation of reperfusion-induced arrhythmias

To assess reperfusion-induced arrhythmias, epicardial electrocardiograms were recorded and analysis was carried out according to the original Lambeth conventions.

3.6. Biochemical analyses

To assess the delayed effect of cardioprotective LPS and rapid ventricular pacing on myocardial peroxynitrite formation, the level of cardiac 3-nitrotyrosine, a well-known maker of peroxynitrite was measured by enzyme-linked immunosorbent assay. The precursors of peroxynitrite, $O_2^{\bullet-}$ and $NO^{\bullet}$ were also evaluated. $O_2^{\bullet-}$ was measured by lucigenin-enhanced chemiluminescence assay or dihydroethidium staining. Spin trapping followed by electron paramagnetic resonance spectroscopy was used to assess the level of $NO^{\bullet}$. To reveal the possible source of cardiac $O_2^{\bullet-}$ and $NO^{\bullet}$ induced by low-dose LPS, activity of XOR and Ca^{2+}-dependent and -independent NOS enzyme activities were measured in the heart.

The activation of ERK1/2, Akt and STAT3 pro-survival kinases was assessed by determining the phosphorylation rate of these proteins based on standard Western blot analysis, to reveal the possible role of RISK and SAFE pathways in the mechanism of alternative conditionings.

3.7. Statistical analysis

Data were analyzed by use of Student's unpaired *t*-test, one-way analysis of variance (ANOVA), two-way ANOVA or Fisher's exact test as appropriate. Differences were considered significant at $p < 0.05$.

4. Results

4.1. Low-dose endotoxin pretreatment improves post-ischemic cardiac function and LDH release: the late cardioprotective effect is verified

Cardiac performance was measured in isolated hearts subjected to global ischemia 24 h after *in vivo* LPS (*S. typhimurium*; 0.5 mg/kg ip.) or saline injection, in order to verify the late cardioprotective effect of low-dose endotoxin. There was no difference in animal weight and heart wet weight between the control and LPS-pretreated groups. Cardiac function was deteriorated in both experimental groups during reperfusion after global ischemia.

The post-ischemic aortic flow, coronary flow, cardiac output, heart rate, left ventricular developed pressure, and the maximum and minimum of its first derivatives ($\pm dp/dt_{max}$) were decreased, while left ventricular end-diastolic pressure was increased compared to the pre-ischemic values. However, post-ischemic decline of aortic flow, cardiac output, left ventricular developed pressure, and $+dp/dt_{max}$ was significantly improved by LPS-pretreatment. Coronary flow, $-dp/dt_{max}$, left ventricular end-diastolic pressure and heart rate were not affected significantly by low-dose LPS-pretreatment after the ischemia.

At the beginning of reperfusion after a global ischemia, LDH release was markedly increased in the control group. In contrast, low-dose LPS pretreatment prevented the post-ischemic LDH release.

4.2. LPS pretreatment enhances myocardial 3-nitrotyrosine formation, $O_2^{\bullet-}$ and $NO^{\bullet}$ production, XOR and NOS activity

To assess the delayed effect of cardioprotective LPS on myocardial peroxynitrite formation, the level of cardiac free 3-nitrotyrosine was measured. Low dose of LPS significantly enhanced the formation of myocardial 3-nitrotyrosine 24 h after the *in vivo* administration.

In order to elucidate the source of enhanced cardiac peroxynitrite formation induced by low-dose LPS, $O_2^{\bullet-}$ and $NO^{\bullet}$, the precursors of peroxynitrite were measured. The cardiac levels of both $O_2^{\bullet-}$ and $NO^{\bullet}$ were significantly increased in LPS-pretreated hearts.

To reveal the possible source of increased $O_2^{\bullet-}$ and $NO^{\bullet}$ levels induced by low-dose LPS, activity of XOR and NOS enzymes were measured. The activity of XOR and Ca^{2+}-independent-NOS was significantly enhanced in LPS-pretreated hearts without affecting the Ca^{2+}-dependent-NOS activity.

4.3. LPS-pretreatment results in enhanced phosphorylation of STAT3, indicating activation of SAFE pathway

In order to elucidate the possible downstream targets of low-dose LPS, the activations of ERK1/2, Akt and STAT3 (members of RISK and SAFE pathways) were investigated 24 h after LPS-pretreatment. Low-dose LPS significantly enhanced cardiac STAT3 phosphorylation and non-significantly increased Akt phosphorylation without affecting phosphorylation of ERK1/2.

4.4. The duration of reperfusion-induced ventricular tachycardia and/or fibrillation is associated with decreased infarct size

To address the question whether there is an association between the duration of reperfusion-induced ventricular tachyarrhythmias and infarct size, a meta-analysis was done. Meta-analysis of six separate studies previously performed in our laboratory using the same experimental protocol (i.e. isolated rat hearts subjected to ischemia/reperfusion) showed that the presence of VT, VF, or VT+VF with a total duration of longer than 60 s in the first 10 min of reperfusion was associated with a markedly decreased infarct size, respectively.

4.5. Rapid ventricular pacing exerts a cardioprotective effect: limits the infarction and reperfusion-induced arrhythmias

In order to assess the possible cardioprotective effect of rapid ventricular pacing, the extent of myocardial infarction (infarct size and LDH release) was measured and reperfusion-induced arrhythmias were analyzed.

Infarct size normalized to area at risk was significantly decreased by short periods of rapid ventricular pacing, similarly to ischemic postconditioning. The post-ischemic LDH release was significantly reduced by rapid ventricular pacing. Ischemic postconditioning also reduced LDH release; however, the difference did not reach the level of statistical significance.

Short periods of rapid ventricular pacing decreased the incidence of reperfusion-induced VT without having a significant effect on VF. In contrast, the incidence of VT and VF was not affected significantly by ischemic postconditioning in our present study.

There was no difference in animal weight, heart wet weight, baseline heart rate, and coronary flow (baseline, beginning of ischemia, end of reperfusion) between the experimental groups. In contrast to ischemic postconditioning, coronary flow at the onset of reperfusion was not changed by short periods of rapid ventricular pacing compared to ischemia/reperfusion control.

4.6. Peroxynitrite is likely involved in rapid ventricular pacing induced-postconditioning

To obtain some mechanistic insight into the beneficial effect of rapid ventricular pacing, cardiac 3-nitrotyrosine and $O_2^{\bullet-}$ were measured at the 7th min of reperfusion following the 30 min index ischemia.

Postconditioning induced by rapid ventricular pacing significantly increased cardiac 3-nitrotyrosine level (a marker of peroxynitrite formation), similarly to ischemic postconditioning. Moreover, the peroxynitrite precursor $O_2^{\bullet-}$ was mildly, but significantly elevated in both postconditioning groups.

To further prove that the postconditioning maneuvers induce nitrative stress, cardiac 3-nitrotyrosine was measured after the postconditioning stimuli applied following normoxic perfusion without index ischemia. The application of brief ischemia/reperfusion cycles or periodic rapid ventricular pacing increased the cardiac formation of 3-nitrotyrosine in the absence of index ischemia.

4.7. Phosphorylation rate of ERK1/2, Akt and STAT3 proteins were not changed in rapid ventricular pacing-induced cardioprotection

To elucidate the possible downstream targets of postconditioning induced by rapid ventricular pacing, RISK and SAFE pathways were investigated.

Rapid ventricular pacing non-significantly enhanced Akt phosphorylation after the index ischemia at the beginning of reperfusion without affecting phosphorylation of ERK1/2 and STAT3, similarly to ischemic postconditioning.

4.8. Repeated brief periods of rapid ventricular pacing increased STAT3 phosphorylation in the absence of index ischemia

The possible effect of both ischemic postconditioning and rapid ventricular pacing protocols (i.e. application of brief ischemia/reperfusion or rapid ventricular pacing) on myocardial RISK and SAFE pathways was also examined in the absence of preceding index ischemia. Applying short periods of rapid ventricular pacing protocol increased STAT3 phosphorylation in normoxic perfusion without index ischemia, in contrast to brief cycles of ischemia/reperfusion. Phosphorylation of Akt and ERK1/2 was not affected significantly by any of the interventions in the absence of index ischemia.

5. Discussion and conclusions

In our present work, we examined LPS-induced late preconditioning and rapid-ventricular pacing-induced postconditioning, two distinct alternative cardioprotective approaches of ischemic pre- and postconditioning, focusing on their effects on myocardial peroxynitrite, RISK and SAFE pathways.

New findings

- low-dose LPS-pretreatment enhances the myocardial peroxynitrite marker, 3-nitrotyrosine formation
- low-dose LPS-pretreatment results in increased phosphorylation of STAT3, indicating activation of SAFE pathway
- rapid endogenous ventricular arrhythmias in the early phase of reperfusion are associated with decreased infarct size
- short periods of rapid ventricular pacing performed at the early phase of reperfusion attenuate reperfusion injury and induce postconditioning
- peroxynitrite may be involved in the rapid ventricular pacing-induced postconditioning
- RISK and SAFE pathways are not activated in mechanism of rapid ventricular pacing-induced cardioprotection

Ischemic preconditioning is a widely used method to protect the heart against ischemia/reperfusion injury; however, the approach is invasive so it is limited to use as a preventive intervention in daily life. Instead, pharmacological preconditioning is a non-invasive way to confer protection, thereby having a great preventive and therapeutic potential in the field of cardiovascular diseases. Low-dose LPS-pretreatment induces pharmacological late preconditioning and enhances cardiac peroxynitrite formation 24 h after the treatment by stimulating cardiac $O_2^{\bullet-}$ and $NO^{\bullet}$ production through XOR and Ca^{2+}-independent-NOS enzymes. Activation of STAT3 before a lethal ischemia may play a role in the beneficial effect of endotoxin-induced delayed preconditioning.

Our meta-analysis revealed that longer than 60 s reperfusion-induced VT/VF was associated with decreased infarct size. Interpretation of these results is difficult since causality was not examined in these studies. A possible explanation for the results of our meta-analysis is that the size of infarction affects the occurrence of sustained VT and/or VF, while another possibility is that longer tachyarrhythmias at the beginning of reperfusion somehow attenuate infarct development. To the best of our knowledge, this latter approach has not been

investigated in the literature, and therefore we tested whether exogenous application of controlled tachycardia induced by rapid ventricular pacing at the onset of reperfusion, as a novel postconditioning method, can elicit cardioprotection.

Application of short periods of rapid ventricular pacing at the onset of reperfusion beneficially affects essential components of reperfusion injury: the infarct size and reperfusion-induced ventricular arrhythmias. In addition, rapid ventricular pacing increases peroxynitrite formation, which likely plays a role in triggering cardioprotection similarly to ischemic postconditioning. Nevertheless, rapid ventricular pacing-induced postconditioning seems to be independent of RISK and SAFE pathways, and further research is needed to elucidate downstream mechanisms. Since rapid ventricular pacing exerted a similar cardioprotective effect to ischemic postconditioning, we feel that rapid ventricular pacing-induced postconditioning may serve as an alternative experimental model of ischemic postconditioning. Moreover, rapid ventricular pacing could be performed in more controlled manner than applying brief ischemia/reperfusion cycles in ischemic postconditioning, which is an important technical advantage compared to the classic method.

Taken together, peroxynitrite may be somehow involved in both alternative cardioprotective approaches. The role of RISK and SAFE pathways seems to be not clear and partly different in these alternative conditionings.

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