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Ischemic postconditioning: brief ischemia during reperfusion converts persistent ventricular fibrillation into regular rhythm

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Abstract

Objectives: Brief episodes of myocardial ischemia-reperfusion employed during reperfusion after a prolonged ischemic insult may attenuate the total ischemia-reperfusion injury. This phenomenon has been termed ischemic postconditioning. In the present study, we studied the possible effect of postconditioning on persistent reperfusion-induced ventricular fibrillation (VF) in the isolated rat heart model. **Methods:** Isolated Langendorff-perfused rat hearts ($n = 46$) were subjected to 30 min of regional ischemia and reperfusion. The hearts with persistent VF ($n = 11$) present after 15 min of reperfusion were then randomly assigned into one of the two groups: (1) control hearts ($n = 6$), in which perfusion was continued without intervention; (2) postconditioned hearts ($n = 5$) subjected to 2 min of global ischemia followed by reperfusion. Left ventricular pressures, heart rate, coronary flow, and electrogram were monitored throughout the experiment. **Results:** Conversion of VF into regular rhythm was observed in all hearts subjected to postconditioning. Regular beating was maintained by all postconditioned hearts during the subsequent reperfusion. None of the hearts in the control group had normal rhythm at the end of the experiment. At the end of reperfusion, the left ventricular developed pressure was lower in beating postconditioned hearts compared to the hearts that did not develop persistent VF. **Conclusions:** Ischemic postconditioning possesses strong antiarrhythmic effect against persistent reperfusion-induced tachyarrhythmias. Postconditioning may be an interesting, novel adjunct strategy to protect the heart. © 2004 Elsevier B.V. All rights reserved.

Keywords: Heart; Ischemia; Reperfusion; Ventricular fibrillation; Postconditioning

1. Introduction

The preconditioning phenomenon was introduced in 1986 by Murry et al. [1] who found that several brief episodes of ischemia-reperfusion prior to a prolonged ischemic insult significantly decreased infarct size. Intensive research has shown that a preconditioning response may be elicited by a variety of chemical and physical stimuli [2,3]. Whereas the classic preconditioning response disappears after a few hours, a delayed preconditioning or second window of protection appears after 24–48 h [4,5]. Finally, it has been shown that ischemic preconditioning may elicit a protective response in other organs: remote preconditioning [6,7].

Recently, it has been shown that the heart can be effectively protected from reperfusion injury not only with preconditioning, but also with brief episodes of ischemia during the early reperfusion period following a pronounced ischemic insult: ischemic postconditioning [8]. In a dog model postconditioning limits infarct size, reduces tissue edema and polymorphonuclear neutrophil accumulation in the area at risk myocardium, and improves endothelial function [8]. It has been suggested that postconditioning is as effective as preconditioning in limiting infarct size and preserving postischemic endothelial function [8].

Another aspect of ischemia-reperfusion injury is reperfusion arrhythmias [9]. We hypothesized that postconditioning might convert serious reperfusion arrhythmias defined as persistent ventricular fibrillation (VF) into regular rhythm. In the present study this hypothesis was

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investigated in the isolated, perfused rat heart subjected to regional ischemia.

2. Materials and methods

All experiments were performed in accordance with the 'Guide for the Care and Use of Laboratory Animals' (publication no. [NIH] 85-23) and were approved by the institutional ethical committee of the St. Petersburg I.P. Pavlov Federal Medical University.

2.1. Isolated heart perfusion

Male Wistar rats weighing 250–300 g used throughout the experiments received 60 mg/kg of pentobarbital sodium and 1000 units of heparin intraperitoneally. The thoracic cavity was opened by a wide transdiaphragmal bilateral incision, the hearts were quickly excised, arrested in ice-cold heparinized saline, and immediately mounted on the Langendorff apparatus by the aortic root. A modified Krebs–Henseleit solution consisting of (in mM) 11 glucose, 118 NaCl, 4.7 KCl, 3.0 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 0.5 EDTA, and 25 NaHCO₃ was equilibrated with 95% O₂ and 5% CO₂ and the pH maintained at about 7.4. The whole system was heated to 37 °C by means of water jacketing. Perfusion pressure was constant at 70 mmHg. The heart was suspended in a thermostatic chamber (37 °C).

To induce regional ischemia, the proximal length of left coronary artery was localized between left atrial appendage and the right ventricular outflow tract followed by the passage of 6–0 polypropylene suture around the major trunk of the left coronary artery or its prominent branches. The thread then was made into an overhand knot, an occluder, and two more threads were tied to the main knot (releasers) as described by Himori et al. [10]. Global ischemia was achieved by clamping the inflow tubing.

2.2. Assessment of cardiac function

A fluid-filled latex balloon connected to a transducer (Baxter, USA) with tubing was inserted into the left ventricle via the mitral valve. Balloon volume was adjusted to give a left ventricular end-diastolic pressure of 5–10 mmHg at the beginning of the experiment. Left ventricular developed pressure (LVDP) was calculated as the difference between left ventricular systolic and end-diastolic pressures. A stainless steel electrode was attached to the left auricle for unipolar electrogram recording relative to the metal aortic cannula. Total coronary flow (CF) was measured by timed collection of perfusate dripping from the right heart into a graduated cylinder. The intraventricular pressures, electrogram, heart temperature, and heart rate (HR) were displayed continuously using cardiomonitor (Hellige, Germany). The parallel registration of the intraventricular pressure and electrogram

was done with chart recorder. Analyses of arrhythmia incidence were carried out in accordance with the Lambeth Conventions [11].

2.3. Experimental protocol and exclusion criteria

Forty-six hearts underwent the following protocol: after 20 min of stabilization, LCA was occluded for 30 min followed by 30 min of reperfusion. No heart had VF during ischemia. VF during reperfusion persisting until the 15th min of reperfusion was referred to as persistent VF. At this time point hearts that developed persistent VF were randomly assigned to one of the two groups: (1) controls ($n = 6$), reperfusion was continued for 15 min without any intervention; (2) postconditioning ($n = 5$), global ischemia was induced for 2 min followed by 13 min of reperfusion. The measurements of LVDP, HR and CF were made at baseline (i.e. at the end of stabilization), after 15 min of regional ischemia, and after 15 and 30 min of reperfusion.

Any heart with an HR less than 250 beats/min, or a CF more than 18 ml/min or less than 8 ml/min at the end of stabilization was excluded from the study. Hearts failing to develop left ventricular systolic pressure of more than 85 mmHg when the end-diastolic pressure was kept less than 10 mmHg were also excluded.

2.4. Statistical analysis

Differences in functional data at each time point were evaluated using one-way analysis of variance (ANOVA), followed by a Scheffe's posthoc test. Categorical data, i.e. VF incidence and appearance of regular rhythm, were compared with Fisher's test. All functional data are presented as mean \pm SD, and P -values less than 0.05 were considered significant.

3. Results

3.1. Ventricular fibrillation

The primary end-point of this study was persistent reperfusion-induced VF. In the model used, VF reproducibly occurred during early reperfusion and appeared either as a transient, self-limiting episode or as a persistent event without spontaneous conversion into a normal rhythm. Forty-six hearts were totally entered in the study. Thirty-five hearts showed only transient, self-limiting episodes of ventricular tachyarrhythmias lasting from the start of reperfusion to 8–12th min of reperfusion. Eleven hearts developed persistent VF during reperfusion and were included for the analysis of postconditioning effects. During 120 s of postconditioning ischemia, the conversion of VF and some heart beating were evident in all the hearts from electrogram and left ventricular

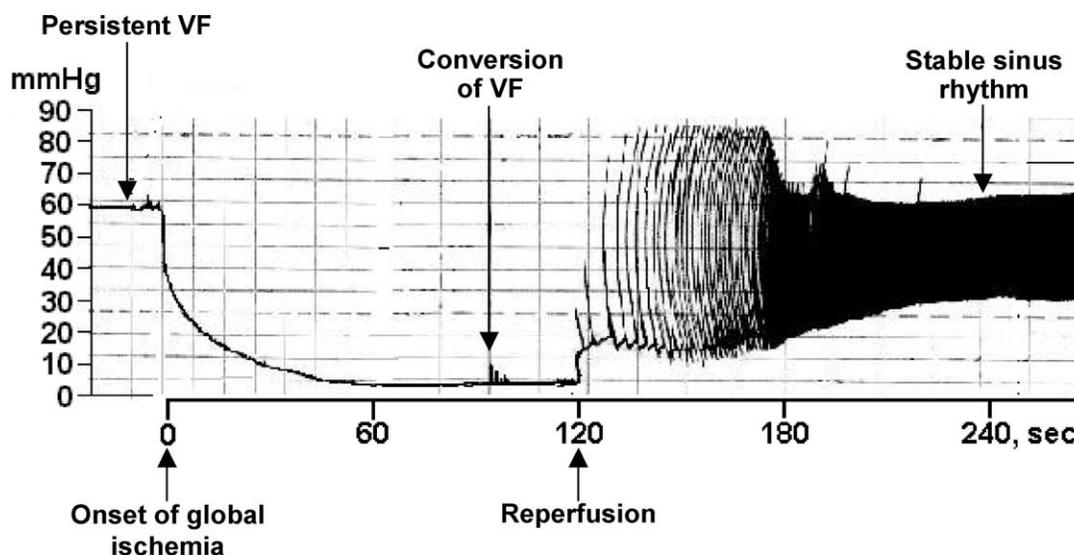


Fig. 1. A representative recording of left ventricular pressure showing the effect of postconditioning on persistent reperfusion-induced ventricular fibrillation (VF).

pressure recording, respectively (Fig. 1). During the first 60–80 s after the start of reperfusion, the rhythm was not regular and multiple ventricular premature beats were registered. However, stable regular rhythm started within 2 min of reperfusion, and all postconditioned hearts maintained regular rhythm during the rest of the observation period. In all control hearts VF continued until the end of reperfusion.

3.2. Left ventricular developed pressure

The baseline values of LVDP were comparable between groups (Table 1). LVDP was reduced during regional ischemia in all groups. However, during ischemia the reduction in LVDP tended to be smaller in non-persistently fibrillating hearts ($P = 0.086$) when compared with the rest of the hearts indicating a possible link between the effects of ischemia and the incidence of persistent VF. At the end of reperfusion, LVDP was lower in the postconditioned hearts as compared to non-persistently fibrillating hearts ($P = 0.048$; Table 1).

3.3. Coronary flow and heart rate

The differences in baseline values of CF between groups could be due to chance (Table 1). During regional ischemia CF was reduced in all groups. CF reduction during ischemia was less in the group of non-persistently fibrillating hearts ($P = 0.064$ vs. persistently fibrillating). At the end of the experiment, CF was significantly higher in the postconditioned hearts when compared to the controls ($P = 0.0024$). Furthermore, at the end of reperfusion CF in postconditioned hearts was not different from CF in non-persistently fibrillating hearts. There were no significant intra- or inter-group differences in HR (Table 1).

4. Discussion

The present study demonstrates that postconditioning by a single brief episode of global ischemia can effectively terminate persistent reperfusion-induced VF and convert it into normal rhythm. During reperfusion after postconditioning ischemia, the regular rhythm was maintained stable.

Table 1

Hemodynamic parameters in the non-persistently fibrillating hearts, control and postconditioned persistently fibrillating Langendorff-perfused rat hearts

	Non-persistently fibrillating hearts (n = 35)	Persistently fibrillating hearts (n = 11)	
		Control (n = 6)	Postconditioned (n = 5)
<i>Baseline</i>			
LVDP	111 ± 6	105 ± 8	108 ± 6
HR	272 ± 21	287 ± 18	276 ± 15
CF	12.5 ± 2.7	13.8 ± 2.5	12.2 ± 1.9
<i>15 min RI</i>			
LVDP	70 ± 10	56 ± 7	62 ± 8
HR	258 ± 22	254 ± 25	242 ± 18
CF	7.1 ± 1.5	6.2 ± 1.2	5.9 ± 1.0
<i>15 min reperfusion</i>			
LVDP	83 ± 12	VF	VF
HR	271 ± 14	VF	VF
CF	11.2 ± 1.4	8.4 ± 1.8	7.5 ± 1.3
<i>30 min reperfusion</i>			
LVDP	86 ± 10	VF	72 ± 9 [†]
HR	268 ± 12	VF	252 ± 15
CF	10.8 ± 1.8	5.3 ± 0.8	10.3 ± 2.1*

Data are given as mean ± SD. LVDP, left ventricular developed pressure (mmHg); HR, heart rate (beats/min); CF, coronary flow (ml/min); RI, regional ischemia. * $P = 0.0024$ vs. control; [†] $P = 0.048$ vs. non-persistently fibrillating hearts.

To our knowledge this is the second paper ever on the postconditioning phenomenon and the first on dealing specifically with serious arrhythmias.

Rhythm disturbances are one of the most important manifestations of myocardial reperfusion-induced injury. Reperfusion tachyarrhythmias may be a cause of sudden death in acute myocardial infarction [12] and may interfere with the recovery of heart function after cardioplegic arrest [13]. The mechanisms of reperfusion arrhythmias are complex and incompletely understood [14]. Among others, free radical formation and accentuation of preexisting heterogeneity of refractoriness occurring during reperfusion may be of primary importance [15,16]. One can assume that reintroduction of ischemia attenuates the formation of reactive oxygen species in the reperfused myocardium and thus interrupts one of the mechanisms of arrhythmia perpetuation. The study performed by Zhao et al. [8] demonstrated that postconditioning was associated with lower plasma levels of malondialdehyde, a product of lipid peroxidation. Furthermore, it has been shown in this study that tissue content of superoxide as revealed by dihydroethidium staining was significantly less in the area at risk myocardium of postconditioned dogs. Taken together, these results suggest reduced production of reactive oxygen species and attenuation of lipid peroxidation as possible mechanisms of the postconditioning phenomenon. On the other hand, global ischemia may make myocardium more electrically homogenous and, therefore, affect such a mechanism of arrhythmia as re-entrant circuits. However, exact mechanisms underlying the described effect remain to be investigated.

For this first study on postconditioning we performed global ischemia to avoid any mechanical interference with the delicate rat heart in the very sensitive and vulnerable period of reperfusion, as such maneuvers might in itself be arrhythmogenic. On the other hand, we have previously shown that reperfusion arrhythmias can be mechanically converted in the isolated perfused rat heart [17]. One limitation of this study is the small number of hearts with persistent fibrillation. However, this is compensated by the clear picture of the effect: with postconditioning all five hearts with persistent fibrillation were converted compared to none among the six control hearts. Furthermore, the present study does not provide any information about protection of cardiac function evaluated by systolic or diastolic function, but it demonstrates protection against arrhythmias, which is one aspect of reperfusion injury. Another limitation is the lack of examination on possible molecular and electrophysiologic mechanisms. Finally, global ischemia in the isolated rat heart studying general principles may be far from the cardiac surgery scenario. Further studies should include more clinically relevant models.

In 1994 Grech and Ramsdale [18] reported that reinflation of the balloon during percutaneous transluminal coronary angioplasty terminated idioventricular rhythm

caused by myocardial reperfusion and restored sinus rhythm. The authors suggested that this effect might be secondary to reinterruption of supply of oxygenated blood. This observation may be a clinical parallel to our experimental data, and also suggest that regional ischemia may be as effective as global ischemia. This is also the case in the pioneering study by Zhao and co-authors [8].

Postconditioning may become an alternative, or perhaps even an adjunct to preconditioning strategy to improve myocardial protection. However, it should be noted that the effect of postconditioning is directed specifically against reperfusion damage to the myocardium while preconditioning is known as a means of both ischemic and reperfusion injury prevention. Preconditioning is traditionally believed to be the most effective weapon against ischemic cell death, i.e. necrosis. How the relation of preconditioning to postconditioning will be, and if they act differently on the various aspects of reperfusion injury remains to be investigated.

Preconditioning is a powerful cardioprotective tool. However, it is only applicable in situations where we know that the ischemic insult is going to happen, such as cardioplegic arrest. However, there are still major limitations in the use of ischemic preconditioning in cardiac surgery [19], because there are serious contraindications to repeatedly clamp and declamp the ascending aorta at a time when one rather prefers surgical techniques with no-touch of the aorta. So far pharmacological preconditioning is not a part of clinical practice. Postconditioning has the advantage of being a way to influence and modify ischemia-reperfusion injury after it has occurred. This may open up a therapeutic alternative in situations of unexpected and uncontrolled ischemic injury, for instance in the situation where complications occur during surgery, making a simple procedure into a complicated one, and making aortic cross-clamping longer than anticipated. Furthermore, by studying the molecular mechanisms of postconditioning novel pharmacological strategies of myocardial protection may emerge.

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References

- [1] Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986;74:1124–36.

- [2] Yellon DM, Downey JM. Preconditioning the myocardium: from cellular physiology to clinical cardiology. *Physiol Rev* 2003;83: 1113–51.
- [3] Domenech R, Macho P. Non-ischemic myocardial preconditioning. *Mol Cell Biochem* 1998;186:201–3.
- [4] Yellon DM, Baxter GF. A second window of protection or delayed preconditioning phenomenon: future horizons for myocardial protection? *J Mol Cell Cardiol* 1995;27:1023–34.
- [5] Bolli R. The late phase of preconditioning. *Circ Res* 2000;87:972–83.
- [6] Gho BC, Schoemaker RG, Van den Doel MA, Duncker DJ, Verdouw PD. Myocardial protection by brief ischemia in noncardiac tissue. *Circulation* 1996;94:2193–200.
- [7] Przyklenk K, Darling CE, Dickson EW, Whittaker P. Cardioprotection outside the box—the evolving paradigm of remote preconditioning. *Basic Res Cardiol* 2003;98:149–57.
- [8] Zhao ZQ, Corvera JS, Halkos ME, Kerendi F, Wang NP, Guyton R, Vinten-Johansen J. Inhibition of myocardial injury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 2003;285: H579–88.
- [9] Hearse DJ, Bolli R. Reperfusion induced injury: manifestations, mechanisms, and clinical relevance. *Cardiovasc Res* 1992;26:101–8.
- [10] Himori N, Matsuura A. A simple technique for occlusion and reperfusion of coronary artery in conscious rats. *Am J Physiol Heart Circ Physiol* 1989;256:H1719–25.
- [11] Walker MJ, Curtis MJ, Hearse DJ, Campbell RW, Janse MJ, Yellon DM, Cobbe SM, Coker SJ, Harness JB, Harron DW, Higgins AJ, Julian DG, Lab MJ, Manning AS, Northover BJ, Parratt JR, Riemersma RA, Riva E, Russell DC, Sheridan DJ, Winslow E, Woodward B. The Lambeth conventions: guidelines for the study of arrhythmias in ischemia, infarction, and reperfusion. *Cardiovasc Res* 1988;22:447–55.
- [12] Virmani R, Burke AP, Farb A. Sudden cardiac death. *Cardiovasc Pathol* 2001;10:211–8.
- [13] Vaage J, Valen G. Pathophysiology and mediators of ischemia-reperfusion injury with special reference to cardiac surgery: a review. *Scand J Thorac Cardiovasc Surg* 1993;41:1–18.
- [14] Manning AS, Hearse DJ. Reperfusion-induced arrhythmias: mechanisms and prevention. *J Mol Cell Cardiol* 1984;16:497–518.
- [15] Jeroudi MO, Hartley CJ, Bolli R. Myocardial reperfusion injury: role of oxygen radicals and potential therapy with antioxidants. *Am J Cardiol* 1994;73:2B–7B.
- [16] Wit AL, Janse MJ. Reperfusion arrhythmias and sudden cardiac death: a century of progress toward an understanding of the mechanisms. *Circ Res* 2001;89:741–3.
- [17] Kawakami T, Lowbeer C, Valen G, Vaage J. Mechanical conversion of post-ischemic ventricular fibrillation: effects on function and myocyte injury in isolated rat hearts. *Scand J Clin Lab Invest* 1999;59: 9–16.
- [18] Grech ED, Ramsdale DR. Termination of reperfusion arrhythmia by coronary artery occlusion. *Br Heart J* 1994;72:94–5.
- [19] Vaage J, Valen G. Preconditioning and cardiac surgery. *Ann Thorac Surg* 2003;75:S709–14.

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