Published in final edited form as:

J Surg Res. 2010 December ; 164(2): 175–181. doi:10.1016/j.jss.2010.08.050.

Ischemic Postconditioning Does Not Provide Cardioprotection from Long Term Ischemic Injury in Isolated Male or Female Rat Hearts

Daniel S. Lee, B.S.¹, Gregory E. Steinbaugh, Ph.D.¹, Ricardo Quarrie, M.D.¹, Fuchun Yang, M.D., M. A.^{2,3}, Hassan Talukder, M.B.B.S., Ph.D.², Jay L. Zweier, M.D.², and Juan A. Crestanello, M.D.¹

¹Division of Cardiothoracic Surgery, Department of Surgery, and Davis Heart and Lung Research Institute, Ohio State University, Columbus, Ohio, USA

²Division of Cardiovascular Medicine, Department of Internal Medicine, and Davis Heart and Lung Research Institute, Ohio State University, Columbus, Ohio, USA

Abstract

Background—Ischemic postconditioning(PoC) is a cardio-protective strategy in which initial reperfusion is interrupted by episodes of ischemia. It is unclear whether PoC can be achieved in the Langendorff perfused rat heart model. We investigated 1) whether postconditioning occurs in Langendorff perfused rat heart and 2) whether there is a gender specific response to PoC.

Materials and methods—Male/female rat hearts(n=8/group) were subjected to 30 minutes of equilibration, 30 minutes of ischemia, and 120 minutes of reperfusion (CONTROL). PoC was induced by 6 cycles(PoC 6c10s), 3 cycles(PoC 3c10s), or 2 cycles(PoC 2c10s) of 10 second reperfusion/10 second ischemia. Rate pressure product(RPP) and infarct size were measured. Male rats(n=7/group) were subjected *in vivo* to 30 minute left coronary ligation followed by 24 hours of reperfusion(CONTROL) or PoC 6c10s and 24 hours of reperfusion.

Results—Recovery of RPP was 18±4% in male CONTROL vs. 17±2% for 6c10s, 16±1% for 3c10s, and 15±3% for 2c10s. Female CONTROL hearts recovered 25±3% of their RPP vs. 21±2% for 6c10s. Infarct size was 25±3% for male CONTROL vs. 26±3% for 6c10s, 30±2% for 3c10s, 28±1% for 2c10s; and 30±2% for female CONTROL vs. 29±2% in 6c10s. *In vivo* Infarct size for CONTROL and PoC 6c10s was 44±3% and 28±5%, respectively (p<0.05).

Conclusions—In the Langendorff perfused rat hearts, none of the PoC protocols improved myocardial tolerance to ischemia reperfusion injury nor decreased infarct size; however, *in vivo* postconditioning did confer protection. The lack of protection in the isolated hearts was not gender specific.

Address correspondence to: J.A. Crestanello, Division of Cardiothoracic Surgery, Ohio State University 410 W 10th Ave, N816 Doan Hall, Columbus, Ohio 43210-1218. Telephone: (614) 293-8866. Fax: (614) 293-4726. Juan.Crestanello@osumc.edu..

³The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, People's Republic of China

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The authors declare they have no competing financial interests.

Presented at the 5th Annual Academic Surgical Congress. San Antonio, Texas. 2.5.2010.

Keywords

Ischemic postconditioning; cardioprotection; ischemic injury; rat heart myocardium; infarct

Introduction

Ischemic postconditioning (PoC) was first proposed by Zhao and colleagues¹ as an alternative method to ischemic preconditioning (IPC)² to improve myocardial tolerance to ischemia-reperfusion (IR) injury. PoC is a protection strategy in which restoration of myocardial perfusion after ischemia is established in a staged fashion with multiple brief episodes of ischemia interrupting the initial reperfusion period. PoC is an “after the fact” protection strategy and may have greater clinical application in particular in reperfusion after myocardial infarction or cardiac surgery.

PoC has been shown to decrease infarct damage *in vivo*^{3,4,5,6}. However, there are reports that show no benefit from postconditioning *in vivo*^{5,7}. The isolated crystalloid perfused heart is a time proven model for the study of ischemia reperfusion injury and its mechanisms². It is unclear whether PoC protection can be achieved in the isolated rat heart. There also has been controversy in terms of gender specific protection associated with PoC. It has been demonstrated that females of different species have improved tolerance to IR injury. Mechanisms underlying this gender specific tolerance to IR injury are thought to involve the sex hormone estrogen^{8,9,10,11}.

The purpose of this study was to investigate the effect of postconditioning on the crystalloid perfused isolated rat hearts and to determine whether there is gender specific response to PoC in the isolated rat heart. PoC was induced by different protocols in male and female rat hearts and mechanical function parameters and myocardial infarct size were measured. The PoC protocols utilized did not improve myocardial tolerance to ischemia reperfusion, nor decrease myocardial infarct size in crystalloid perfused isolated male and female rat hearts. We speculate that humoral or blood factors may play an important role on the induction of PoC.

Materials and Methods

Isolated Heart Preparation

Male and female Sprague-Dawley rats weighing between 275-350 g (Harlan Laboratories, Indianapolis, IN) were housed in a quiet environment and fed a standard diet. Rats were anaesthetized with sodium pentobarbital (60 mg/kg intraperitoneally) (Ovation Pharmaceuticals, Deerfield, IL) and heparin sodium (500 U intraperitoneally) (APP Pharmaceuticals, LLC, Schaumburg, IL) in accordance with the “Guide for the Care and Use of Laboratory Animals” (NIH Publication No 86-23, revised 1996), the “Public Health Service Policy on Humane Care and Use of Laboratory Animals” (National Institutes of Health, revised 2002), and the “Animal Welfare Act” (United States Drug Administration, revised 2007). Hearts were excised and perfused in a Langendorff apparatus at a constant aortic pressure of 76 mmHg with oxygenated (95% O₂, 5% CO₂) Krebs-Henseleit buffer containing (in mM) 118 NaCl, 4.6 KCl, 1.17 KH₂PO₄, 1.17 MgSO₄, 1.16 CaCl₂, 23 NaHCO₃, and 5.3 glucose, pH 7.4, at 37°C. A latex balloon was inserted in the left ventricle through the mitral valve and attached to a pressure transducer (COBE, Lakewood, CO) for monitoring of ventricular function. Left ventricular end diastolic pressure was set at 10 mm Hg. Hearts were maintained at 37°C throughout the experiment by immersion in a water-jacketed non-gassed perfusate bath. Global non flow ischemia was created by a stopcock located immediately above the aorta. Heart rate and left ventricular developed pressure

(LVDP: peak systolic minus end diastolic pressure) were recorded throughout the experiment. Rate pressure product (RPP) was calculated by multiplying the heart rate and left ventricular developed pressure. Coronary flow was measured using a TS410 transmit time tubing flowmeter (Transonic Systems Inc., Ithaca, NY). Data were continuously recorded using a PowerLab Chart v4.2 (AD Instruments Inc., Milford, MA) and a Dell GenuineIntel x86 Family 6 Model Stepping 6 computer (Dell Computer Corp., Round Rock, TX).

Postconditioning Protocols

Hearts (n=8/group) were assigned to the CONTROL or Postconditioning groups (figure 1). In the control group (CONTROL) hearts were subjected to 30 min of equilibration, followed by 30 min of global normothermic ischemia, and 120 min of reperfusion. In the postconditioning groups, postconditioning was induced after 30 min of equilibration, and 30 min of global normothermic ischemia, by either a) 6 cycles of 10 second reperfusion and 10 second ischemia (PoC 6c10s), b) 3 cycles of 10 second reperfusion and 10 second ischemia (PoC 3c10s), and c) 2 cycles of 10 second reperfusion and 10 second ischemia (PoC 2c10s). Hearts were then reperfused for a total of 120 minutes.

Infarct Size Measurement

Infarct measurements were made using 2,3,5-triphenyltetrazolium chloride (TTC, Fluka, Milwaukee, WI). Briefly, ventricles were removed from the Langendorff apparatus at end reperfusion and placed at -20°C for 30 min for hardening. Ventricles were cut with a rat heart slicer (Zivic Instruments, Pittsburgh, PA) into 2 mm thick pieces. Slices were incubated with TTC (45 mM in PBS) solution at 37°C for 15 minutes and then stored overnight in 10% formalin solution (Sigma-Aldrich, Inc., St. Louis, MO) for color enhancement. Infarct size measurements were taken using Metamorph v.7.1.2.0 software (MDS Analytical Technologies, Toronto, CN) for clear visualization of infarct (white) versus healthy (red). Percent (%) infarct of slice was calculated by determining the infarct area (white) of the heart slice divided by total area of the heart slice. The following formula was used to determine total infarct size for each heart: the sum of % infarct of slice * weight of slice (in g) divided by the sum of weight of slice (in g).

In vivo Postconditioning Protocol and Measurement of Myocardial Infarction

Male Sprague-Dawley (Harlan, Indianapolis, IN) rats aged 8-10 wks were randomly allocated into 2 groups (n=7/group): CONTROL, subjected to 30 min of ischemia and 24 hours of sustained reperfusion; and postconditioning (PoC 6c10s), subjected to 30 min of ischemia followed by 6 cycles of 10 second reperfusion and 10 second ischemia, and then 24 hours of sustained reperfusion. Animals were provided standard rat chow and water ad libitum.

Rats were anesthetized with intraperitoneal administration of Ketamine (74.8 mg/kg) and Xylazine (10 mg/kg). A neck mid-line incision was made, and the trachea was exposed and intubated with a cannula connected to a rodent ventilator (model 683, Harvard Apparatus, Inc.). The rat was ventilated with room air at 90–95 breaths/min and 0.2 ml/tidal volume. Body temperature was maintained at $37.0\text{--}37.5^{\circ}\text{C}$ using a heating pad. A left thoracotomy was performed on the fourth intercostal space, followed by a pericardiectomy. The left atrial appendage was retracted to reveal the location of the left coronary artery. The left anterior descending coronary artery was encircled with 6-0 silk suture to form a slipknot 2.0 mm below the level of the tip of the normally positioned left atrial appendage and a small piece PE-10 tube was placed in-between for convenient release upon reperfusion. Left anterior descending coronary artery occlusion was confirmed by the dramatic change in color (red to pallor) and restricted ventricular motion. Reperfusion was initiated by releasing the slipknot

and removing the tube, and was confirmed by the appearance of epicardial hyperemic response. The suture was kept in position for next day re-ligation and staining, and the chest wall was closed in layers.

After 24 hours of reperfusion, rats were euthanized, hearts were rapidly excised, flushed with heparinized PBS via aorta, then incubated with 1% 2,3,5-triphenyltetrazolium chloride (TTC, Sigma) for 5 min for demarcation of viable and nonviable myocardium within the area at risk (AAR). Left anterior descending coronary artery was reoccluded and 10% phthalo-blue was injected via the aorta for visualization of the non-risk (non-ischemic, NI) region. The heart was frozen, serially sectioned with a slicer (2 mm thickness), and fixed in 10% formalin. Both sides of each slice were photographed to measure the AAR and infarct area (IA) by computerized planimetry (with image analysis software Meta Vue, version 6.0).

Statistical Analysis

Data was expressed as mean $\pm$ SEM. Time dependent *in vitro* data were compared using Repeated Measures two way ANOVA and the Holm-Sidak post hoc analysis. Group comparisons were made using two way ANOVA. *In vivo* data were analyzed by two-tailed Student's *t*-test for unpaired data from different experiments. A *p* value less than 0.05 was considered to be statistically significant.

Results

Mechanical Function in Isolated Male Hearts

Recovery of left ventricular developed pressure (LVDP) at end reperfusion was $23 \pm 3\%$ for CONTROL male hearts vs. $22 \pm 2\%$ for PoC 6c10s (*P* = NS); $20 \pm 2\%$ for PoC 3c10s (*P* = NS); and $21 \pm 3\%$ for PoC 2c10s (*P* = NS), Fig. 2A. Recovery of rate pressure product (RPP) also showed a similar trend to LVDP: $18 \pm 4\%$ for CONTROL vs. $17 \pm 2\%$ for PoC 6c10s (*P* = NS); $16 \pm 1\%$ for PoC 3c10s (*P* = NS); and $15 \pm 3\%$ for PoC 2c10s (*P* = NS), Fig. 2B. There was no significant difference in left ventricular end diastolic pressure nor coronary flow between the CONTROL group and the different PoC groups during reperfusion (Fig. 2C and D).

Mechanical Function in Isolated Female Hearts

Recovery of left ventricular developed pressure (LVDP) during reperfusion was $28 \pm 3\%$ in CONTROL female rat hearts vs. $25 \pm 3\%$ in PoC 6c10s (*P* = NS), Fig. 3A. Recovery of rate pressure product (RPP) was $25 \pm 3\%$ in CONTROL vs. $21 \pm 2\%$ for PoC 6c10s, (*P* = NS), (Fig. 3B). Left ventricular end diastolic pressure and coronary flow during reperfusion was similar between the CONTROL group and the PoC group in female hearts (Fig 3C and 3D).

Infarct Size in Isolated Male Hearts

Infarct size at end reperfusion was similar in the CONTROL group and the three PoC groups in male rat hearts: $25 \pm 3\%$ of the area at risk in CONTROL vs. $26 \pm 3\%$ in PoC 6c10s (*P* = NS), $30 \pm 2\%$ in PoC 3c10s (*P* = NS), and $28 \pm 1\%$ for PoC 2c10s (*P* = NS), Fig. 4. Figure 4A-D shows representative ventricular slices of CONTROL and PoC hearts showing no difference in infarct size.

Infarct Size in Isolated Female Hearts

In female rat hearts, PoC did not decrease infarct size ($30 \pm 2\%$ of the area at risk in CONTROL vs. $29 \pm 2\%$ in PoC 6c10s, (*P*=NS, Fig 4 and 4E-F).

In vivo Infarct Size in Male Rats

The percentage of area at risk (AAR) in the left ventricle (LV) for CONTROL and PoC 6c10s was similar. ($58 \pm 3\%$ and $59 \pm 1\%$, Figure 5). PoC 6c10s significantly reduced infarct size compared to CONTROL: the infarct area (IA) as a percentage of AAR was $44 \pm 3\%$ in CONTROL and $28 \pm 5\%$ in PoC 6c10s ($p < 0.05$).

Discussion

Post conditioning (PoC) is an attractive cardioprotective strategy since it can be applied after the ischemic insult (“after the fact”) and therefore requires no foreknowledge of the ischemic event¹². It has the potential for a wider clinical application than preconditioning. Studies have suggested that male hearts subjected to postconditioning can be protected after a 30 min period of ischemia^{6,13,14,15,16} and can decrease infarct damage *in vivo*^{3,4,5,6}. Although protection by postconditioning has been reproduced in a large variety of other *in vivo* models^{3,4,5,6}, it is unclear whether it can be induced in the isolated crystalloid perfused rat heart. The lack of cardioprotection observed in crystalloid perfused rat heart may be gender specific or related to the postconditioning protocol^{8,9,10,11,17,18}. This study investigated the effect of different postconditioning protocols on the crystalloid perfused isolated rat hearts. Experiments were conducted in male and female rat hearts to determine whether there is gender specific response to PoC in that model. Left ventricular function parameters and infarct size were examined. We observed, in our isolated crystalloid perfused rat heart, that 1) PoC does not protect the heart from ischemia-reperfusion injury and 2) that lack of protection is not gender specific. In addition, we demonstrated that using a similar protocol as in the *in vitro* model, PoC 6c10s, postconditioning significantly decreases myocardial infarct size *in vivo* compared to CONTROL.

It is unclear whether the number and length of ischemia reperfusion cycles used to induce PoC affects the cardioprotection. Previous studies have shown that cardioprotection can be achieved using a variety of protocols. Yang et al. demonstrated that six cycles of 30 seconds of reperfusion / 30 seconds of ischemia provided a more robust protection than four cycles of 30 seconds of reperfusion / 30 seconds of ischemia⁶ in rabbits. However, Zhao demonstrated a decrease in infarct size with PoC induced by 3 cycles of 30 second reperfusion¹ in dogs. In humans, 4 one minute episodes of PoC decreases creatine kinase released during percutaneous coronary intervention for myocardial infarction⁴. Several other PoC protocols did not trigger cardio-protection: 4 cycles of 10 seconds of reperfusion/10 seconds of reocclusion, 4 cycles of 20 seconds of reperfusion/20 seconds of reocclusion, 8 cycles of 30 seconds of reperfusion/30 seconds of reocclusion, and 20 cycles of 10 seconds of reperfusion/10 seconds of reocclusion in rats⁷. We used three postconditioning protocols to test whether there is a correlation between the number of ischemia-reperfusion cycles used to induce PoC and cardioprotection in isolated male hearts. The present study showed that PoC does not confer cardioprotection on isolated male rat hearts as manifested by lack of improvement in the mechanical function parameters examined regardless of the postconditioning protocol used.

Several authors have demonstrated that women and females from different species have improved tolerance to myocardial ischemia-reperfusion injury that results in improved survival^{17,18} compared to males. Estrogens lower LDL and fibrinogen levels, raise HDL levels, and have antioxidant properties. Estrogens also enhance flow-mediated coronary vasodilatation^{8,9,10,11,17}. Endogenous estrogen may up-regulate the threshold of postconditioning-induced cardioprotection in female rats *via* JNK and TNF expression^{9,19}. In addition, estrogen has many beneficial effects on the coagulation system, and on the endothelium decreasing inflammation and adhesion¹⁸. It has been speculated that gender differences in tolerance to ischemia reperfusion injury are due to changes in the mitogen-

activated protein kinase (MAPK) signaling pathway. It is upregulated in males and downregulated in females⁹. When examining the effect of gender on postconditioning, there were mixed results: Dow et al. has observed that myocardial infarct size was not reduced in an *in vivo* rat study in either males or females⁷. However, Penna et al. demonstrated that postconditioning reduced infarct size and post-ischemic systolic dysfunction after 30 minutes of global ischemia only in female rats²⁰. Our experiments failed to demonstrate a gender difference in the induction of postconditioning in the isolated rat heart.

There is a lack of blood cells and cytokines in the isolated crystalloid perfused rat heart of the Langendorff model^{21,22,23} whereas these blood components do exist in the *in vivo* studies which may explain the absence of protection by postconditioning in the isolated hearts. Our model is a non-recirculating model where the effluent from the heart is discarded. Therefore, the myocardium is not exposed to potential protective “humoral mediators” released by the postconditioning ischemia reperfusion episodes. Those “humoral mediators” released by ischemia reperfusion may induce cardioprotection. One example is remote preconditioning where ischemia reperfusion of an organ distant from the heart induces cardioprotection²³. It is postulated that these “humoral mediators” released by the remote ischemic organ elicit cardioprotection when they reach the heart. Also exposing the heart to effluents of another heart subjected to ischemia reperfusion induced cardioprotection²². Postulated “humoral mediators” implicated are norepinephrine²², adenosine²², and protein kinase C²³. Also, higher oxygen levels in the Langendorff system may lead to increased production of oxygen radicals compared to *in vivo* which may result in increased damage to the isolated rat hearts.

We conclude that while cardioprotection is present *in vivo* postconditioning, the postconditioning protocols investigated do not confer cardioprotection in the isolated crystalloid perfused male or female rat hearts. The isolated crystalloid perfused rat heart is not an adequate model for the study of postconditioning.

Acknowledgments

This work was supported by NIH grants HL63744, HL65608 and HL38324, and a Grant from the Thoracic Surgery Foundation for Research and Education.

References

1. Zhao Z-Q, Corvera JS, Halkos ME, Kerendi F, Wang N-P, Guyton RA, Vinten-Johansen J. Inhibition of myocardial injury by ischemic postconditioning by reperfusion: comparison with ischemic preconditioning. *Am. J. Physiol. Heart Circ. Physiol* 2003;285:H579–H588. [PubMed: 12860564]
2. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986;74:1124–36. [PubMed: 3769170]
3. Laskey WK. Brief repetitive balloon occlusion enhance reperfusion during percutaneous coronary intervention for acute myocardial infarction: a pilot study. *Cath. Cardio. Interven* 2005;65:361–367.
4. Staat P, Rioufol G, Piot C, Cottin Y, Cung TT, L'Huillier I, Aupetit J-F, Bonnefoy E, Finet G, André-Fouët X, Ovize M. Postconditioning the human heart. *Circulation* 2005;112:2143–2148. [PubMed: 16186417]
5. Ma X, Zhang X, Li C, Luo M. Effect of postconditioning on coronary blood flow velocity and endothelial function and LV recovery after myocardial infarction. *J. Interven. Cardiol* 2006;19:367–375.
6. Yang X-M, Philipp S, Downey JM, Cohen MV. Postconditioning's protection is not dependent on circulating blood factors or cells but involves adenosine receptors and requires PI3-kinase and guanylyl activation. *Basic Res. Cardiol* 2005;100:57–63. [PubMed: 15614590]
7. Dow J, Kloner RA. Postconditioning does not reduce myocardial infarct size in an *in vivo* regional ischemia rodent model. *J. Cardio. Pharma. Therapeu* 2007;12:153–163.

8. Cavasin MA, Tao Z, Menon S, Yang X-P. Gender differences in cardiac function during early remodeling after acute myocardial infarction in mice. *Life Sci* 2004;75:2181–2192. [PubMed: 15325844]
9. Wang M, Baker L, Tsai BM, Meldrum KK, Meldrum DR. Sex differences in the myocardial inflammatory response to ischemia-reperfusion injury. *Am. J. Physiol. Endocrinol. Metab* 2005;288:E321–E326. [PubMed: 15367393]
10. Hodis HN, Mack WJ, Lobo RA, Shoupe D, Sevanian A, Mahrer PR, Selzer RH, Liu C-R, Liu C-H, Azen SP. Estrogen in the prevention of atherosclerosis. A randomized, double-blind, placebo-controlled trial. *Ann. Intern. Med* 2001;135:939–953. [PubMed: 11730394]
11. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *New Eng. J. Med* 2009;23:1801–1811.
12. Crisostomo PR, Wairiuko GM, Wang M, Tsai BM, Morrell ED, Meldrum DR. Preconditioning versus postconditioning: mechanisms and therapeutic potentials. *Am. Coll. Surg* 2006;202:797–812.
13. Darling CE, Jiang R, Maynard M, Whittaker P, Vinten-Johansen J, Przyklenk K. Postconditioning via stuttering reperfusion limits myocardial infarct size in rabbit hearts: role of ERK 1/2. *Am. J. Physiol. Heart Circ. Physiol* 2005;289:H1618–H1626. [PubMed: 15937101]
14. Argaud L, Gateau-Roesch O, Raissy O, Loufouat J, Robert D, Ovize M. Postconditioning inhibits mitochondrial permeability transition. *Circ* 2005;111:194–197.
15. Galagudza M, Kurapeev D, Minasian S, Valen G, Vaage J. Ischemic postconditioning: brief ischemia during reperfusion converts persistent ventricular fibrillation into regular rhythm. *Euro. J. Cardio. Surg* 2004;25:1006–1010.
16. Kin H, Zhao Z-Q, Sun H-E, Wang N-P, Corvera JS, Halkos ME, Kerendi F, Guyton RA, Vinten-Johansen J. Postconditioning attenuates myocardial ischemia-reperfusion injury by inhibiting events in the early minutes of reperfusion. *Cardio. Res* 2004;62:74–85.
17. Rossouw JE. Hormones, genetic factors, and gender differences in cardiovascular disease. *Cardio. Res* 2002;53:550–557.
18. Baker L, Meldrum KK, Wang M, Sankula R, Vanam R, Raiesdana A, Tsai B, Hile K, Brown JW, Meldrum DR. The role of estrogen in cardiovascular disease. *J. Surg. Res* 2003;115:325–344. [PubMed: 14697301]
19. Pitcher JM, Wang M, Tsai BM, Kher A, Nelson NT, Meldrum DR. Endogenous estrogen mediates a higher threshold for endotoxin-induced myocardial protection in females. *Am. J. Physiol. Regul. Integr. Comp. Physiol* 2006;290:R27–R33. [PubMed: 16150837]
20. Penna C, Tullio F, Merlino A, Moro F, Raimondo S, Rastaldo R, Perrelli MG, Mancardi D, Pagliaro P. Postconditioning cardioprotection against infarct size and post-ischemic systolic dysfunction is influenced by gender. *Basic Res. Cardiol* 2009;104:390–402. [PubMed: 19030912]
21. Serejo FC, Rodrigues LF Jr. da Silva Tavares KC, de Carvalho AC, Nascimento JH. Cardioprotective properties of humoral factors released from rat hearts subject to ischemic preconditioning. *J. Cardiovasc. Pharmacol* 2007;49:214–20. [PubMed: 17438406]
22. Dickson EW, Lorbar M, Porcaro WA, Fenton RA, Reinhardt CP, Gysembergh A, Przyklenk K. Rabbit heart can be “preconditioned” via transfer of coronary effluent. *Heart Circ. Physiol* 1999;277:H2451–H2457.
23. Weinbrenner C, Nelles M, Herzog N, Sárváry L, Strasser RH. Remote preconditioning by infrarenal occlusion of the aorta protects the heart from infarction: a newly identified non-neuronal but PKC-dependent pathway. *Cardio. Res* 2002;55:590–601.
24. Sandritter W, Jestädt R. Triphenyl-tetrazolium (TTC) als Reduktionsindikator zur makroskopischen Diagnose des frischen Herzinfarktes. *Vehr. Dtsch. Ges. Path* 1958;41:165–170.
25. Jestädt R, Sandritter W. Erfahrungen mit der TTC (Triphenyltetrazoliumchlorid) Reaktion für die pathologisch-anatomische Diagnose des frischen Herzinfarktes. *Z. Kreislaufforsch* 1959;48:802–809.
26. Klein HH, Puschmann S, Schaper J, Schaper W. The mechanism of the tetrazolium reaction in identifying experimental myocardial infarction. *Virchows. Arch* 1981;393:287–297.

27. Schwarz ER, Somoano Y, Hale SL, Kloner RA. What is the required reperfusion period for assessment of myocardial infarct size using triphenyltetrazolium chloride staining in the rat? *J. Thrombo. Thrombol* 2000;10:181–187.

Figure 1.

Postconditioning protocols for isolated hearts. Control hearts were subjected to 30 min of equilibration (EQ), 30 min of global normothermic ischemia for 30 min (I), and 90 min of reperfusion (RP). Postconditioning was induced by either 6 cycles of 10 s reperfusion and 10 s of ischemia (PoC 6c10s), 3 cycles of 10 s reperfusion and 10 s of ischemia (PoC 3c10s), or 2 cycles of 10 s reperfusion and 10 s of ischemia (PoC 2c10s).

Figure 2. Recovery of left ventricular developed pressure (A) and rate pressure product (B) in isolated **male hearts** expressed as a percentage of their end equilibration values. (C) Left ventricular end diastolic pressure during reperfusion in mmHg. (D) Coronary flow during reperfusion in ml/min.

Figure 3. Recovery of left ventricular developed pressure (A) and rate pressure product (B) in isolated **female hearts** expressed as a percentage of their end equilibration values. (C) Left ventricular end diastolic pressure during reperfusion in mmHg. (D) Coronary flow during reperfusion in ml/min.

Figure 4.

Left ventricular infarct size in CONTROL and postconditioning isolated rat hearts at end reperfusion.

Infarct size is expressed as a percentage of left ventricle at risk. Representative transversely sectioned heart slices are displayed above: (A) CONTROL in male rats. (B) PoC 6c10s in male rats. (C) PoC 3c10s in male rats. (D) PoC 2c10s in male rats. (E) CONTROL in female rats. (F) PoC 6c10s in female rats. Infarcted areas are seen in white.

Figure 5.

Left ventricular infarct size in CONTROL and PoC 6c10s rat hearts *in vivo* at end reperfusion. (A) Representative transversely sectioned heart slices of CONTROL and PoC 6c10s. Infarcted areas are seen in white and the area not at risk are seen in blue. (B) Bar graph showing the percentage of area at risk (AAR) over left ventricle (LV) and the percentage of infarct area (IA) over AAR. Note that postconditioning decreases infarct size *in vivo*. * $p < 0.05$ vs. CONTROL.