The Effects of Ischemic Postconditioning on Myocardial Function and Nitric Oxide Metabolites Following Ischemia-Reperfusion in Hyperthyroid Rats

Jalal Zaman1,2, Sajjad Jeddi1,2, and Asghar Ghasemi1,2

1Endocrine Physiology Research Center, 2Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran 19395-4763, Iran

Ischemic postconditioning (IPost) could decrease ischemia-reperfusion (IR) injury. It has not yet reported whether IPost is useful when ischemic heart disease is accompanied with co-morbidities like hyperthyroidism. The aim of this study was to examine the effect of IPost on myocardial IR injury in hyperthyroid male rats. Hyperthyroidism was induced with administration of thyroxine in drinking water (12 mg/L) over a period of 21 days. After thoracotomy, the hearts of control and hyperthyroid rats were perfused in the Langendorff apparatus and subjected to 30 minutes global ischemia, followed by 120 minutes reperfusion; IPost, intermittent early reperfusion, was induced instantly following ischemia. In control rats, IPost significantly improved the left ventricular developed pressure (LVDP) and ±dp/dt during reperfusion (p<0.05); however it had no effect in hyperthyroid rats. In addition, hyperthyroidism significantly increased basal NOx (nitrate+nitrite) content in serum (125.5±5.4 μmol/L vs. 102.8±3.7 μmol/L; p<0.05) and heart (34.9±4.1 μmol/L vs. 19.9±1.94 μmol/L; p<0.05). In hyperthyroid groups, heart NOx concentration significantly increased after IR and IPost, whereas in the control groups, heart NOx were significantly higher after IR and lower after IPost (p<0.05). IPost reduced infarct size (p<0.05) only in control groups. In hyperthyroid group subjected to IPost, aminoguanidine, an inducible nitric oxide (NO) inhibitor, significantly reduced both the infarct size and heart NOx concentrations. In conclusion, unlike normal rats, IPost cycles following reperfusion does not provide cardioprotection against IR injury in hyperthyroid rats; an effect that may be due to NO overproduction because it is restored by iNOS inhibition.

Key Words: Hyperthyroidism, Ischemia, Nitric oxidem, Postconditioning, Reperfusion

INTRODUCTION

Ischemic heart disease (IHD) is a major cause of mortality worldwide [1]. Most patients with myocardial infarction (MI) due to coronary artery disease (CAD), also have other risk factors including aging, hypertension, atherosclerosis, diabetes, and thyroid diseases that lead to increased incidence of MI [2]. Decreased coronary blood flow leads to reduction in delivery of blood, oxygen, and other nutrients to the heart, leading to heart ischemia [3]. Regardless of requirement of reperfusion to re-establish the normal func-

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Corresponding to: Asghar Ghasemi, Endocrine Physiology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, No. 24, Parvaneh Street, Velenjak, Tehran 19395-4763, Iran. (Tel) 98-21-22432500, (Fax) 98-21-2241-6264, (E-mail) Ghasemi@endocrine.ac.ir

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Serum total T4 (thyroxine), T3 (triiodothyronine), and TSH (thyroid stimulating hormone) levels in the control and hyperthyroid animals were determined at the end of treatment phase using commercial Elisa kits. Changes in serum T4, the latter using the Srere procedure [18]. The frozen heart samples were also deproteinized using zinc sulfate (15 mg/ml). Serum samples were also deproteinized using zinc sulfate (15 mg/ml) and centrifuged at 10,000 g for 10 min at 4°C. For both serum and tissue samples, a 100 μl of the supernatant was added to a microplate well and 100 μl vanadium (III) chloride (8 mg/ml) was added to each well (for reduction of nitrate to nitrite), followed by addition of 50 μl sulfanilamide (2%) and 50 μl NEDD (N-(1-naphthyl) ethylenediamine dihydrochloride) (0.1%). After 30 min incubation at 37°C, the absorbance was read at 540 nm using the ELISA reader (BioTek, Powerwave XS2). NOx concentrations in serum and tissue samples, a 100 μmol/L of sodium nitrate, respectively. Heart and serum homogenates samples were measured from the linear standard curve established by 0-150 and 0-50 μmol/L of sodium nitrate, respectively. Heart and serum NOx levels are presented as μmol/L. Intra-assay coefficient of variation was 5.2%.

Measurement of NOx

Following 2-hour reperfusion, samples of left ventricle (LV) tissue were immediately frozen in liquid nitrogen and stored at −80°C. NOx contents in serum and myocardium homogenates were determined using the Griess method [20]. Briefly, after homogenization of samples in PBS (phosphate-buffered saline) (1:5, w/v), the homogenates were centrifuged at 15,000 g for 20 min at 4°C. The supernatants were removed from the homogenates and were deproteinized by addition of zinc sulfate (15 mg/ml). Serum samples were also deproteinized using zinc sulfate (15 mg/ml) and centrifuged at 10,000 g for 10 min at 4°C. For both serum and tissue samples, a 100 μl of the supernatant was added to a microplate well and 100 μl vanadum (III) chloride (8 mg/ml) was added to each well (for reduction of nitrate to nitrite), followed by addition of 50 μl sulfanilamide (2%) and 50 μl NEDD (N-(1-naphthyl) ethylenediamine dihydrochloride) (0.1%). After 30 min incubation at 37°C, the absorbance was read at 540 nm using the ELISA reader (BioTek, Powerwave XS2). NOx concentrations in serum and heart homogenates samples were measured from the linear standard curve established by 0-150 and 0-50 μmol/L of sodium nitrate, respectively. Heart and serum NOx levels are presented as μmol/L. Intra-assay coefficient of variation was 5.2%.

Measurement of infarct size

At the end of the reperfusion period, infarct sizes (IS) were determined as described previously [21]. The frozen heart samples were cut into thin slices (∼2-3 mm) and were incubated for 10 min in 1% of 2, 3, 5-triphenyltetrazolium
chloride (TTC) in phosphate buffer solution 20 mM/L, pH 7.4 at 37°C. The slices were immersed in 10% formalin for 24 h to identify viable myocardium as red stained, while necrotic (infarcted) tissue remains unstained (pale gray). The sections were photographed using a digital camera version DV101 (Samsung, Japan). IS was measured by Photoshop CS6 software (version 13) and expressed as percentage of the total area.

Statistical analyses

All data are presented as means±SEM. LVDP, LVEDP, ±dp/dt, IS and NO, values were evaluated by repeated measure ANOVA; a post-hoc Tukey test was applied using SPSS software for comparing means between groups, with p values < 0.05 being considered significant.

RESULTS

CS activity in soleus muscle and thyroid hormone levels (T3, T4) in serum were significantly higher, whereas serum TSH was significantly lower in hyperthyroid rats. In addition, body weight changes in hyperthyroid rats were not significantly different from controls, and ratio of heart weight to body weight was higher in hyperthyroid rats.

Table 1. General Characteristics of animals in the hyperthyroid and control groups

|                     | Control (n=8) | Hyperthyroidism (n=8) |
|---------------------|--------------|----------------------|
| Weight change (g)   | 19.2±3.70    | 15.1±1.4             |
| Heart weight (g)    | 1.02±0.04    | 1.36±0.04*           |
| Heart weight/Body weight (%) | 0.38±0.01    | 0.52±0.01*           |
| T3 (nmol/L)         | 0.76±0.06    | 1.84±0.22*           |
| T4 (nmol/L)         | 49.4±2.30    | 107.7±6.8*           |
| TSH (ng/ml)         | 6.8±0.60     | 1.9±0.6*             |
| Citrate synthase activity (µmol/ml/min) | 1.16±0.30 | 1.82±0.3* |

The data are presented as means±SEM. *p < 0.05 compared with control group.

Table 2. Baseline values of cardiac function in control and hyperthyroid groups

|                     | Control (n=8) | Hyperthyroidism (n=8) |
|---------------------|--------------|----------------------|
| LVEDP (mm Hg)       | 8.5±1.0      | 9.3±1.0              |
| LVDP (mm Hg)        | 94±4.1       | 68±4.0*              |
| +dp/dt (mm Hg/s)    | 2948±81      | 2490±94*             |
| -dp/dt (mm Hg/s)    | 2171±103     | 1575±75*             |
| Heart rate (pulse/min) | 241±8      | 331±7*               |

The data are means±SEM. Left ventricular end diastolic pressure (LVEDP); Left ventricular developed pressure (LVDP), maximum and minimum in left ventricular pressure (+dp/dt); data are means±SE (n=8 rats); *p < 0.05.

Fig. 1. Alterations of cardiac function during reperfusion; (A) Left ventricular developed pressure (LVDP); (B) Maximum increase in left ventricular pressure (+dp/dt); (C) Decrease in left ventricular pressure (-dp/dt); (D) Heart rate; control-ischemia reperfusion (C-IR), C-ischemic postconditioning (C-IPost), hyperthyroid-IR (HP-IR), hyperthyroid-IPost-aminoguanidine (HP-IPost-AG); data are means±SE (n=8 rats); *p < 0.05 significant difference between C-IR and C-IPost groups. †p < 0.05 significant difference between HP-IPost and HP-IPost-AG.
Preischemic hemodynamic values were significantly lower in the hyperthyroid groups than controls (Table 2). When ischemia was induced by the stopping the inflow of the perfusion solution, the LVDP, ±dp/dt, and heart rate rapidly declined and ceased in the isolated hearts.

Post-ischemic ±dp/dt and LVDP decreased significantly in the HP-IR group, compared with the C-IR group following ischemia (30 min) and reperfusion (120 min). IPost significantly improved the ±dp/dt and LVDP in reperfusion phase in the C-IPost group; however, it did not improve ±dp/dt and LVDP in the HP-IPost group. Following IR, post-ischemic ±dp/dt and LVDP increased significantly in the HP-IPost-AG group, compared to the HP-IPost group (Fig. 1).

During the 30 minutes ischemia, hyperthyroid group showed a significant increase in LVEDP (contracture), compared to controls. IPost significantly reduced the LVEDP during reperfusion phase in the C-IPost group; in addition, IPost in combination with AG, significantly reduced the LVEDP during reperfusion phase in the HP-IPost-AG group compared to HP-IPost (Fig. 2).

Heart and serum NOx levels were significantly higher in the hyperthyroid group, compared to the controls. The level of NOx was enhanced significantly in both groups of HP-IR and HP-IPost. IPost had no significant effect on reduction of heart NOx level in the HP-IPost group, although, IPost significantly reduced the IR-induced enhancement in heart NOx of the control group. AG significantly reduced heart NOx levels in hyperthyroid rats subjected to IPost (Fig. 3). There was significant difference in the IS between groups of C-IR and HP-IR animals (47.5±1.9% vs. 64±2.3%). IPost significantly reduced the IS in control group, while it had no effect in the hyperthyroid group; IPost in presence of (Table 1).

Fig. 2. Alterations of LVEDP during IR; left ventricular end diastolic pressure (LVEDP); control-ischemia reperfusion (C-IR); C-ischemic postconditioning (C-IPost), hyperthyroid-IR (HP-IR); hyperthyroid-IPost (HP-IPost) and hyperthyroid-IPost-aminoguanidine (HP-IPost-AG); data are mean±SE (n=8 rats); *p<0.05 significant difference between C-IR and C-IPost. †p<0.05 significant difference between C-IR and HP-IR, and between C-IPost and HP-IPost. ††p<0.05 significant difference between with HP-IPost and HP-IPost-AG.

Fig. 3. The alterations of NOx in heart of control and hyperthyroid groups; control- ischemia reperfusion (C-IR); C-ischemic postconditioning (C-IPost); hyperthyroid-IR (HP-IR); hyperthyroid-IPost (HP-IPost), and hyperthyroid-IPost-aminoguanidine (HP-IPost-AG); data are as mean±SE (n=8 rats); *p<0.05 compared with control group. **p<0.05 compared with C-IR group. #p<0.05 compared with hyperthyroid group. †p<0.05 compared with hyperthyroid group.

Fig. 4. The alterations of infarct size in heart of control and hyperthyroid groups. control- ischemia reperfusion (C-IR); C-ischemic postconditioning (C-IPost); hyperthyroid-IR (HP-IR); hyperthyroid-IPost (HP-IPost), and hyperthyroid-IPost-aminoguanidine (HP-IPost-AG); data are mean±SE (n=6 rats) as present of total area; *p<0.05 compared with control group. **p<0.05 compared with C-IR group. †p<0.05 compared with hyperthyroid group. ††p<0.05 compared with HP-IPost group.
AG significantly reduced the IS in HP-IPost-AG group (60.5±2.04 vs. 47.60±1.72) (Fig. 4).

DISCUSSION

Our findings indicate that hyperthyroidism increase injury induced by IR in rat heart, which may be due to increasing of NO production. IPost provides protective effects against IR injury in control rats, but, has no effect in hyperthyroid rats. Heart hypertrophy increases both the soleus muscle CS activity, and also levels of serum thyroid hormones (T4 and T3), while decreasing TSH levels, showing that hyperthyroidism has been effectively induced.

In our study, preischemic values of LVDP and ±dp/dt were lower in the hyperthyroid rats; the hearts from hyperthyroid rats, exhibited a decreased recovery of LVDP and ±dp/dt following IR indicating that these hearts are vulnerable to IR injury, findings consistent with previous studies [13-15]. It has been reported that chronic T4 administration (21 days) results in increased calcium concentration in cardiac cells [22], which, in turn, causes mitochondrial malfunction, and, consequently cell death [22]. Contradictory to our results, which were on a long-term basis it has been reported that hyperthyroidism provides cardioprotective effects following IR [23,24]; a likely explanation for this difference may be moderately short-term T4 administration used in these studies [24].

In this study, pre-ischemic heart rate was higher in the hyperthyroid group than the controls; in this regard, studies have been shown that hyperthyroidism increases the heart rate through various mechanisms, such as augmented L-type calcium channels [25], elevated activity of Ca2+ ATPase [26], and declined phospholamban [27]. Our results show that hearts from hyperthyroid rats subjected to global ischemia developed ischemic contracture (increasing Ca2+ concentration in heart cells are crucial factors in ischemic contracture [28]. High levels of thyroid hormones can increase the susceptibility of these hearts to IR and may be associated with H2O2 production and mitochondrial dysfunction [15,29,30].

In our study, IPost failed to offer any protection in hearts of hyperthyroid rats, unlike the hearts of control rats, in which it seems that IPost had a cardioprotective effect, via inhibiting mPTP (mitochondrial Permeability Transition Pore). It has been identified that in hypertrophic status during myocardial reperfusion injury, opening of mPTP occurs possibly due to ROS production and mitochondrial Ca2+ overload [31,32]. Therefore, it seems that IPost possibly loses its effectiveness in a hyperthyroid group, a finding similar to those from diabetic rats, where preconditioning and IPost cannot protect the hearts of diabetic rats against IR injury [33,34].

In our study, IPost showed cardioprotective effect in non-hyperthyroid animals by reducing the IS, but failed to reduce IS in hyperthyroid animals. Similar to our results, it has been reported that cardioprotective effects of IPost in reducing IS are abrogated in disease state including metabolic syndrome [35] and diabetes [34,36].

In the current study, hypertrophy occurred along with increasing of heart NO metabolites in the hyperthyroid group; it has been reported that thyroid hormones lead to activation of the inducible NO synthase (iNOS) directly and increasing NO levels [37]. In the hearts of hyperthyroid rats, upregulation of iNOS causes NO overproduction, which is accompanied by elevated oxidative stress [37,38]. Also, other studies have shown that the high production of NO may contribute in cardiacc hypertrophy [39,40]. Similar to our results, increasing of heart NO level in hyperthyroid rats following IR has been previously reported [14,30]. Our results show that hyperthyroidism increases injuries induced by IR in rat hearts, which is possibly due to elevated NO levels. It has been hypothesized that hyperthyroidism could lead to reperfusion injury by increase of NO production, which, in turn increases nitro-oxidative stress [30]. Data shows that the high levels of NO, during the IR stage is possibly an important factor of hyperthyroid-induced cardiomyopathy [14]. The interaction between both NO and free radicals has been shown to increase lipid peroxidation in reperfusion [30]. Thus overproduction of free radicals in hyperthyroidism is associated with reduced antioxidant defense. The studies have shown that the harmful effect of NO on IR is mediated by peroxynitrite [41]. The combination of high level of NO with superoxide can produce peroxynitrite that this is considered a toxic agent in the death of myocardial cells [41,42].

In this study, IPost, significantly diminished NOx in the hearts of control rats, whereas, we observed increasing of NOx in the HP-IPost group. In addition, IPost did not protect the heart from IR injury in hyperthyroid rats. Some investigations have demonstrated that IPost protects the non-diseased rat heart from IR injury, by diminishing of the NO concentration in the heart, subsequent to ischemia phase [41-44]. Therefore, it seems that the increasing of NO and free radicals in hyperthyroidism, causes loss of IPost efficiency in the hearts of hyperthyroid rats; this pattern that NO overproduction by iNOS which prevents cardioprotective effect of IPost in hyperthyroidism has also been reported in diabetic rats [45,46]. It has been shown that iNOS inhibition could restore cardioprotective effects of IPost in diabetes [46]. Similarly in the current study, we showed that IPost in presence of AG, which selectively inhibits iNOS, provide cardioprotection in hyperthyroid rats.

In conclusion, hyperthyroidism increased susceptibility of heart to IR injury and unlike control rats, IPost could not provide protection against this injury due to NO overproduction; cardioprotective effects of IPost in hyperthyroid rats are restored in presence of iNOS inhibition.

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REFERENCES

1. Peart JN, Headrick JP. Clinical cardioprotection and the value of conditioning responses. Am J Physiol Heart Circ Physiol. 2009;296:H1708-1720.
2. Ferdinandy P, Schulz R, Baxter GF. Interaction of cardiovascular risk factors with myocardial ischemia/reperfusion injury, preconditioning, and postconditioning. Pharmacol Rev.
1. Venditti P, Masullo P, Agnisola C, Di Meo S. 
2. Rastaldo R, Pagliaro P, Cappello S, Penna C, Mancardi D, Westerhof N, Losano G. Nitric oxide and cardiac function. 
3. Life Sci. 2000;66:697-708. 
4. Phospholamban protein expression. Regulatory effects on sarcoplasmic reticulum Ca2+-ATPase. 
5. Free Radic Res. 2005;112:454-462. 
6. Ashida K, Katsura T, Saito H, Inui K. Decreased activity and expression of intestinal oligopeptide transporter PEPT1 in rats with hyperthyroidism in vivo. 
7. Pharm Res. 2004;21:969-975. 
8. Liu PM, Donley DA, Bryner RW, Alway SE. Citrate synthase expression and enzyme activity after endurance training in cardiac and skeletal muscles. 
9. J Appl Physiol (1985). 2003;94:555-560. 
10. Belaïdi E, Béguin PC, Levy P, Ribault C, Godin-Ribault D. Delayed myocardial preconditioning induced by cobalt chloride in the rat. 
11. HF1α and INOS involvement. Fandom Clin Pharma. 
12. Mol 2012:26:454-462. 
13. Miranda KM, Espey MG, Wink DA. A rapid, simple spectro-photometric method for simultaneous detection of nitrate and nitrite. 
14. Nitric Oxide. 2001;5:62-71. 
15. Sont HM, Jain MR, Mehta AA. Mechanism(s) Involved in Carbon Monoxide-releasing Molecule-2-mediated Cardioprotection During Ischaemia-reperfusion Injury in Isolated Rat Heart. 
16. Indian J Pharm Sci. 2012;74:281-291. 
17. Shacklebey D, Asadomihini A, Hesami S, Vaezi M, Shahidi S. Cardioprotective effect of diazepam on ischemia-reperfusion isolated hyperthyroid rat heart. 
18. Turk J Biol. 2012;36:598-605. 
19. Pantos C, Malliopoulos V, Varonas DD, Cokkinos DV. Thyroid hormone and phenotypes of cardioprotection. Basic Res Cardiol. 
20. 2004;99:101-120. 
21. Pantos C, Malliopoulos V, Mourouzis I, Thempeyioti A, Paisiz I, Dinopoulos A, Saranteas T, Xinaris C, Cokkinos DV. Hyper-thyroid hearts display a phenotype of cardioprotection against ischemic stress: a possible involvement of heat shock protein HSP 72. 
22. Kreuzberg U, Theissenn S, Schicha H, Schroder F, Meihoorn U, de Vivie ER, Boknik P, Neumann J, Grohe C, Herzig S. Single-channel activity and expression of atrial L-type Ca2+ channels in patients with latent hyperthyroidism. Am J Physiol Heart Circ Physiol. 2000;278:H723-731. 
23. Arruda AP, Da-Silva WS, Carvalho DP, De Meis L. Hyper-thyroidism increases the uncoupled ATPase activity and heat production by the sarcoplasmic reticulum Ca2+-ATPase. 
24. Biochem J. 2003;375:735-740. 
25. Kiss E, Jakab G, Kranias EG, Edes I. Thyroid hormone-induced alterations in phospholamban protein expression. Regulatory effects on sarcoplasmic reticulum Ca2+ transport and myocardial relaxation. 
26. Circ Res. 1994;75:245-251. 
27. Segal J, Massalha S, Schwalb H, Merin G, Bornan JB, Uretzky G. Acute effect of thyroid hormone in the rat heart: role of calcium. J Endocrinol. 1996;149:73-80. 
28. Venditti P, Agnisola C, Di Meo S. Effect of ischemia-reperfusion on heart mitochondria from hyperthyroid rats. Cardiovasc Res. 2005;62:76-85. 
29. Masullo P, Venditti P, Agnisola C, Di Meo S. Role of nitric oxide in the reperfusion induced injury in hyperthyroid rat hearts. Free Radic Res. 2000;32:411-421. 
30. Juhászovsz M, Zorov BB, Yaniv Y, Nuss HB, Wang S, Sollett SJ. Role of glycogen synthase kinase-3beta in cardioprotection. 
31. Circ Res. 2009;104:1240-1252. 
32. Kaldiron B, Hermes O, Bar-Tana J. Mitochondrial permeability transition is induced by in vivo thyroid hormone treatment. 
33. Endocrinology. 1996;136:3552-3556. 
34. Yin X, Zheng Y, Zhai X, Zhao X, Cui L. Diabetic inhibition of preconditioning- and postconditioning-mediated myocardial protection against ischemia/reperfusion injury. Exp Diabetes Res. 2012;2012:198048. 
35. Badauldadeh R, Mohammad M, Najafi M, Ahmadzadi N, Farajinia S, Ebrahimi H. The additive effects of ischemic postconditioning and cyclosporine-A on nitric oxide activity and functions of diabetic myocardium injured by ischemia/reperfusion. J Cardiovasc Pharmacol Ther. 2012;17:181-189. 
36. Wagner C, Klotzing I, Strasser BH, Weihbrunner C. Cardioprotection by postconditioning is lost in WOKW rats with metabolic syndrome: role of glycogen synthase kinase 3beta. 
37. J Cardiovasc Pharmacol. 2008;52:430-437. 
38. Przyklenk K, Maynard M, Greiner DL, Whittaker P. Cardioprotection with postconditioning: loss of efficacy in murine models of type-2 and type-1 diabetes. Antioxid Redox Signal. 2011;14:781-790. 
39. Rodríguez-Gómez I, Wangensteen R, Moreno JM, Chamorro V, Osuna A, Vargas F. Effects of chronic inhibition of inducible nitric oxide synthase in hyperthermic rat hearts. Am J Physiol Endocrinol Metab. 2005;288:E1252-1257. 
40. Oztay F, Ergin B, Ustunsoy S, Balci H, Kapucu A, Caner M, Demirci C. Effects of coenzyme Q10 on the heart ultrastructure and nitric oxide synthesis during hyperthermia. Chin J Physiol. 2007;50:217-224. 
41. Kuzman JA, Vogelsang KA, Thomas TA, Gerdes AM. L- Thyroxine activates Akt signaling in the heart. J Mol Cell Cardiol. 
42. 2005;39:251-258. 
43. Araujo AS, Schenkel P, Enwezor AT, Fernandes TR, Partata WA, Lluesy S, Ribeiro MF, Khaiper N, Singal FK, Belló-Klein A. The role of redox signaling in cardiac hypertrophy induced by experimental hyperthyroidism. J Mol Endocrinol. 2008;41:423-430. 
44. Fan Q, Yang X, Liu Y, Wang LF, Liu SH, Ge YG, Chen ML,
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Wang W, Zhang LK, Irwin MG, Xia Z. Postconditioning attenuates myocardial injury by reducing nitro-oxidative stress in vivo in rats and in humans. Clin Sci (Lond). 2011;120:251-261.

42. Ferdinandy P, Schulz R. Nitric oxide, superoxide, and peroxy-nitrite in myocardial ischaemia-reperfusion injury and preconditioning. Br J Pharmacol. 2003;138:532-543.

43. Gao Q, Hu JF, Yu Y, Jiang CR, Guan SD, Li ZH. The role of nitric oxide in ethanol postconditioning induced cardioprotection. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2012;28:9-13.

44. Csonka C, Szilvássy Z, Fülöp F, Páli T, Blasig IE, Tosaki A, Schulz R, Ferdinandy P. Classic preconditioning decreases the harmful accumulation of nitric oxide during ischemia and reperfusion in rat hearts. Circulation. 1999;100:2260-2266.

45. Nagareddy PR, Xia Z, McNeill JH, MacLeod KM. Increased expression of iNOS is associated with endothelial dysfunction and impaired pressor responsiveness in streptozotocin-induced diabetes. Am J Physiol Heart Circ Physiol. 2005;289:H2144-H2152.

46. Wang T, Yao S, Xia Z, Irwin MG. Adiponectin: mechanisms and new therapeutic approaches for restoring diabetic heart sensitivity to ischemic post-conditioning. Front Med. 2013;7:301-305.