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Cardioprotective effect of postconditioning against ischemia-reperfusion injury is lost in heart of 8-week diabetic rat

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Abstract. Although ischemic preconditioning (IPC) and ischemic postconditioning (IPost) result in protection against ischemia-reperfusion (I/R) injury in healthy hearts, pathological conditions such as diabetes can modify the protective effects of IPC and IPost. There are a few studies concerning the effect of IPost only in diabetic hearts which have similar or decreased tolerance to I/R injury. In the present study we investigated the effects of IPost in diabetic hearts which had increased tolerance to I/R injury. Isolated hearts from control and diabetic rats were subjected to global ischemia (40 min) followed by reperfusion (40 min). IPost was induced by six cycles (10 s) of reperfusion and ischemia after the global ischemia. After I/R, cardiac recovery in diabetic hearts was better than that in control hearts. IPost did not produce any further protection in the diabetic hearts whereas it resulted in a significant recovery in the control hearts. Similarly, the decreased troponin I (TnI) levels of diabetic hearts did not change after IPost. However, IPost significantly lowered the increase in TnI levels of control hearts. In conclusion, these results show that IPost can not produce a further protection in the hearts of 8-week diabetic rats which have increased tolerance to I/R injury.

Key words: Ischemia — Reperfusion — Heart — Postconditioning — Rat

Introduction

Cardiac and vascular complications of diabetes (i.e. cardiomyopathy, micro- and macro-angiopathy) can contribute to ischemic heart disease (Hayat et al. 2004) which is also closely linked with myocardial ischemia-reperfusion (I/R) injury (Dubrey et al. 1994). Therefore, a great deal of clinical studies has demonstrated that diabetic hearts are more susceptible to I/R injury (Zuanetti et al. 1993; Abbud et al. 1995). However, experimental findings about the tolerance of diabetic hearts to I/R injury are controversial. In this regard, some data showing that I/R injury in diabetic heart was increased, decreased or unchanged has been discussed in a few reviews (Feuvray and Lopaschuk 1997; Paulson 1997; Whittington et al. 2012).

Myocardial reperfusion is essential for viability of ischemic myocardium. However, the effects of reperfusion are complex and can increase ischemic injury of the heart with own detrimental effects, resulting in functional impairment, increased arrythmia and infact size (Piper et al. 2004; Buja 2005; Yadav et al. 2010). Both ischemic preconditioning (IPC) and ischemic postconditioning (IPost) are cardioprotective interventions described for the first time in dogs by Murry et al. (1986) and Zhao et al. (2003), respectively. IPC is multiple brief I/R episodes applied before sustained ischemia (Murry et al. 1986; Yadav et al. 2010). IPost can be induced by both repetitive short periods of reperfusion and non-lethal ischemia applied just before the prolonged reperfusion at the end of the sustained ischemia (Sasaki et al. 2007; Pinheiro et al. 2009; Buchholz et al. 2014). A large number of studies have shown that these classic pre- and post-conditioning salvage myocardium from I/R injury thereby resulting in decreased infact size and arrhythmia, and better recovery in cardiac contractile functions (Murry et al. 1986; Zhao et al. 2003; Sasaki et al. 2007; Pinheiro et al. 2009; Yin et al. 2012; Buchholz et al. 2014).

Although detailed knowledge about beneficial effects of IPC and IPost against I/R injury has been well documented

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in healthy animal and human studies (Murry et al. 1986; Yellon et al. 1993; Zhao et al. 2003; Staat et al. 2005; Sasaki et al. 2007; Pinheiro et al. 2009; Yin et al. 2012; Buchholz et al. 2014), there is only limited information as to whether IPC and IPost can produce same cardioprotection in pathological conditions such as diabetes (Miki et al. 2012; Yin et al. 2012). The results of those studies indicate that diabetes generally inhibits IPC- and IPost-mediated cardioprotection against I/R injury (Miki et al. 2012; Yin et al. 2012). The majority of experiments relating to IPC have been carried out in the diabetic hearts which have increased sensitivity to I/R injury (Kersten et al. 2000; Kristiansen et al. 2004; Gross et al. 2007; Hotta et al. 2010). However, Ravingerova et al. (2000) indicated that susceptibility to ischemia-induced arrhythmias was lower in 1-week diabetics but higher in 9-week diabetics. Interestingly, they also showed that the IPC elicited a reduction in incidence of arrhythmias only in the 9-week diabetic hearts. On the other hand, it has been found that IPC could further protect type 2 diabetic rat hearts which had significantly smaller infarcts after I/R injury (Liu et al. 1993; Tsang et al. 2005).

As to IPost, the beneficial effect of this intervention has also been found to be blunted in streptozotocin (STZ) diabetic hearts which had decreased tolerance to I/R injury (Ren et al. 2011). Moreover, the loss of cardioprotective effect of IPost was also reported in hearts from type-1 or type-2 diabetic rodents which had similar infact size after I/R injury (Przylenk et al. 2011; Badalzadeh et al. 2012). To the best of our knowledge, there is no study to evaluate the effect of IPost in diabetic rat hearts which have increased tolerance to I/R injury. In our previous study, we found that the hearts of 8-week diabetic rat had better recovery after I/R injury (Altunkaynak et al. 2009). This study, therefore, was aimed to investigate the cardioprotective effect of IPost in diabetic hearts which had increased tolerance to I/R injury.

Materials and Methods

Animals

Animal procedures of the present study were approved by Ankara University Animal Care and Use Committee (Decision no: 2010-54-271). 8-week-old male Sprague Dawley rats weighing 300–350 g were housed in a room (23 ± 2°C and 12-h light: 12-h darkness cycle), and the rats had free access to food and tap water.

Induction of experimental diabetes

Diabetes was induced by a single tail vein injection of STZ (45 mg/kg, Sigma) in citrate buffer (0.1 M, pH: 4.5). Age-matched control rats received an injection of citrate buffer alone. After 72 hours of injection of STZ or citrate buffer, tail vein blood glucose levels were measured using glucometer (Accu-ChekGo, Roche) with test strips (Accu-ChekGo, Roche). Rats with blood glucose above 300 mg/dl were considered as diabetic. We also measured body weights, blood glucose levels, food and water consumptions of both groups at the end of 8 weeks.

Perfusion technique of isolated rat heart

Hearts were isolated from control and diabetic rats 8 weeks after the induction of diabetes. Under the ether anesthesia, hearts were isolated and rapidly mounted on the Langendorff apparatus. Each heart was perfused retrogradely with Krebs-Henseleit (K-H) buffer composed of (in mM): 120 NaCl, 4.8 KCl, 1.25 $CaCl_2$, 1.25 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, and 10 glucose (37°C, pH = 7.4). The perfusion buffer was gassed continuously with a 95% O_2 and 5% CO_2 mixture. After excision of atriums, the hearts were stimulated at 300 beats/min with using Grass S44 stimulator (Grass Instrument. Inc., Quincy, MA, USA). A water-filled latex balloon connected to a pressure transducer was inserted into the left ventricle and the left ventricular end-diastolic pressure (LVEDP) was adjusted at 5–10 mmHg at the beginning of each experiment. The hearts were firstly perfused at constant pressure (60–65 mmHg) and coronary flow rates were detected with a flowmeter (Transonic Systems Inc., Quincy, MA, USA). Then, the detected flow rate for each heart was set through a pump (Masterflex Model:77200-12, Cole-Parmer, USA), and the hearts were perfused with their own flow rates. During the preischemic period, the coronary flow rates (ml/min/g) which were corrected by heart weights were not significantly different between control (10.3 ± 0.5 ml/min/g) and diabetic (9.2 ± 0.5 ml/min/g, $p > 0.05$) hearts. The left ventricle function was assessed by recording the left ventricular developed pressure (LVDP), and the rates of pressure development (+dP/dt) and pressure decay (–dP/dt) with on-line through an analog–digital interface (MP100 Biopac Systems Inc., Santa Barbara CA). The LVDP was calculated from the difference between the left ventricular peak pressure and LVEDP. The measurements were made at the end of the stabilization period for baseline values and also at the end of the 40 min reperfusion period.

The isolated rat hearts were divided into four groups: Control ischemia-reperfusion group (Control I/R, $n = 6$), Diabetic ischemia-reperfusion group (Diabetic I/R, $n = 6$), Control postconditioning group (Control IPost, $n = 6$) and Diabetic postconditioning group (Diabetic IPost, $n = 8$). In the ischemia-reperfusion groups, after stabilization period for 20 min, the hearts from diabetic and control rats were subjected to 40 min of global ischemia followed by 40 min of reperfusion. In the postconditioning groups, the post-

conditioning was induced by six cycles of reperfusion and ischemia, both with equal length of 10 s immediately after the prolonged ischemia and before the sustained reperfusion period.

Coronary effluent was also collected at the end of the reperfusion and rapidly stored at −80°C. Troponin I (TnI) levels were determined using the the Access AccuTnI assay (Beckman Coulter Inc.) described previously (Venge et al. 2001; Zhang et al. 2012).

Statistical analysis

The results of tests were expressed as the number of observations (n), mean ± standard error of mean. The results of the homogenity (Levene's Test) and normality tests (Shapiro Wilk) were used to decide which statistical methods to apply in the comparison of the study groups. According to those tests results, the Student's t-test for parametric test and Mann-Whitney U test for non-parametric test were used to compare two groups. To compare three or more groups, the two-factor factorial design analysis of variance was applied. In order to detect multiple comparisons "adds syntax" function through "Bonferroni-Dunn test" was used. All statistical analyses were performed with the SPSS software (SPSS Ver. 17.0; SPSS Inc., Chicago IL, USA). $p < 0.05$ was considered statistically significant.

Results

General characteristics of control and diabetic rats at the end of the 8 weeks are shown in Table 1. As expected, the blood glucose levels were significantly increased and body weights were decreased in diabetic rats compared to control rats ($p < 0.05$). A marked polyphagia and polydipsia also occurred in diabetic rats (Table 1).

Although preischemic LVEDP values of hearts from control and diabetic rats were not different, preischemic LVDP, +dP/dt and –dP/dt values of the hearts were found to be significantly lower in diabetic rats compared to control rats, indicating the development of diabetes-induced cardiac failure (Figure 1).

Table 1. The blood glucose level, body weight, food and water consumption of control (n = 12) and diabetic (n = 14) rats at the end of the 8 week

Parameter	Group	
	Control	Diabetic
Body weight (g)	413 ± 8	250 ± 14*
Blood glucose level (mg/dl)	98 ± 4	358 ± 18*
Food consumption (g/day)	20 ± 1	36 ± 2*
Water consumption (ml/day)	46 ± 4	177 ± 15*

* $p < 0.05$ *vs.* Control group.

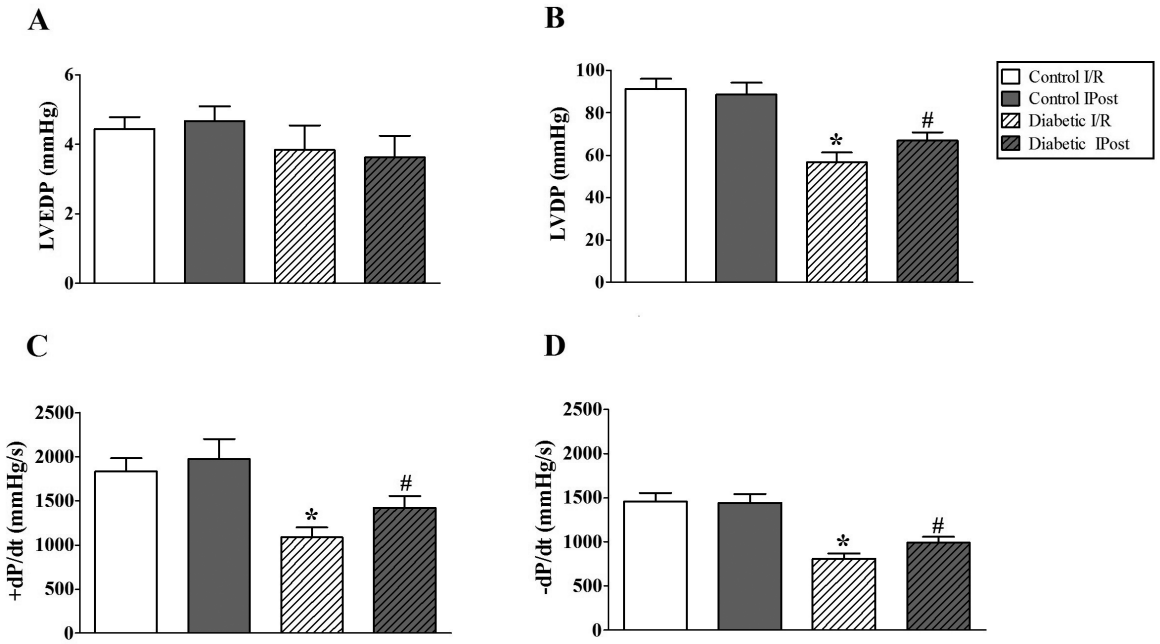

Figure 1. Preischemic cardiac function parameters: LVEDP (**A**), LVDP (**B**), +dP/dt (**C**), –dP/dt (**D**) of isolated hearts from Control I/R (n = 6), Control IPost (n = 6), Diabetic I/R (n = 6) and Diabetic IPost (n = 8) groups. * $p < 0.05$ *vs.* Control I/R; # $p < 0.05$ *vs.* Control IPost. LVEDP, left ventricular end-diastolic pressure; LVDP, left ventricular developed pressure; +dP/dt, the rate of pressure development; –dP/dt, the rate of pressure decay; I/R, ischemia/reperfusion; IPost, postconditioning.

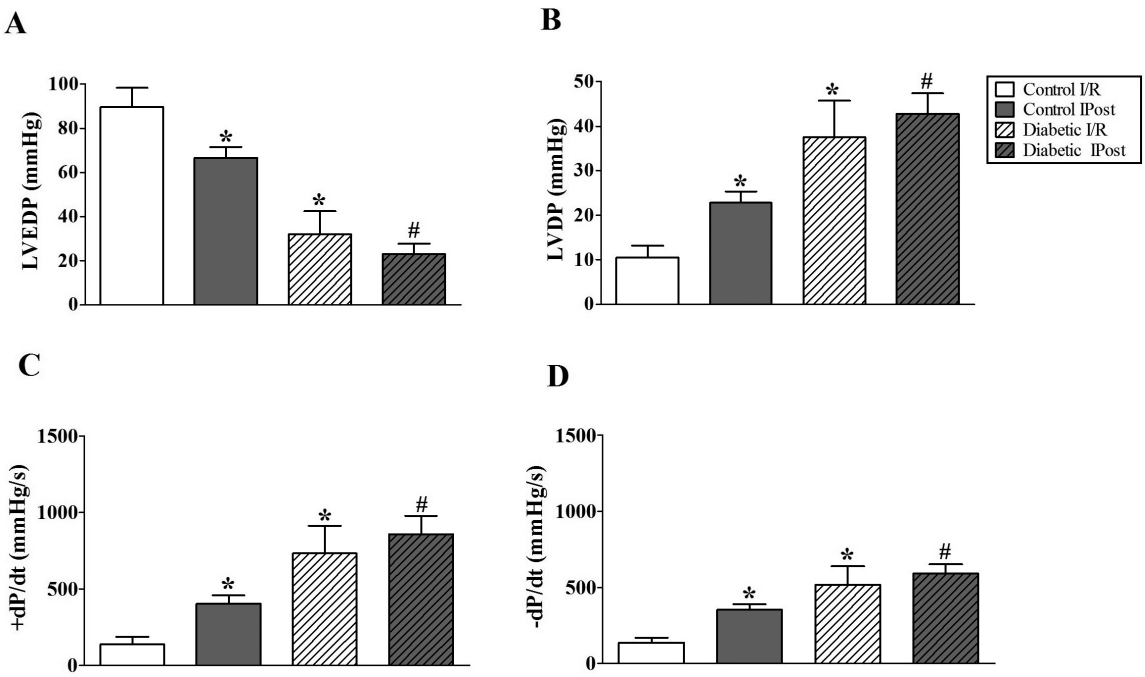

Figure 2. Cardiac function parameters: LVEDP (**A**), LVDP (**B**), +dP/dt (**C**), –dP/dt (**D**) of isolated hearts from Control I/R (n = 6), Control IPost (n = 6), Diabetic I/R (n = 6), Diabetic IPost (n = 8) groups. * $p < 0.05$ *vs.* Control I/R; # $p < 0.05$ *vs.* Control IPost. LVEDP, left ventricular end-diastolic pressure; LVDP, left ventricular developed pressure; +dP/dt, the rate of pressure development; –dP/dt, the rate of pressure decay; I/R, ischemia/reperfusion; IPost, postconditioning.

Figure 2 indicates the alterations in measured parameters relating to cardiac functions of hearts from control and diabetic rats at the end of reperfusion. In the Control I/R group, a minor recovery of LVDP, +dP/dt and –dP/dt in conjunction with markedly elevated LVEDP occured. However, diabetic hearts showed a better recovery in the cardiac parameters after I/R injury (Figure 2). To put it another way, hearts of 8-week diabetic rats had increased tolerance to I/R injury than controls. IPost produced a significant improvement in the cardiac function parameters of control hearts but not diabetic hearts ($p < 0.05$, Figure 2).

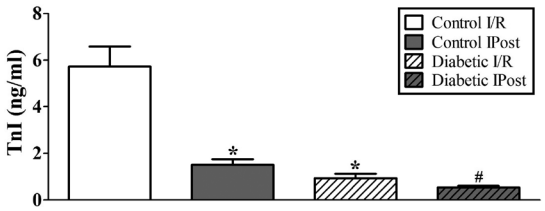

Figure 3. Troponin I (TnI) levels of isolated hearts from Control I/R (n = 6), Control IPost (n = 6), Diabetic I/R (n = 6), Diabetic IPost (n = 8) groups. * $p < 0.05$ *vs.* Control I/R; # $p < 0.05$ *vs.* Control IPost. I/R, ischemia/reperfusion; IPost, postconditioning.

In consistent with the cardiac functions, TnI levels of diabetic hearts were lower than those of control hearts. IPost lowered cardiac TnI levels only in the control hearts. However, IPost did not cause any significant change in the TnI level of the diabetic hearts (Figure 3).

Discussion

A large body of evidence shows that IPost results in cardioprotection against I/R injury in healthy condition. However, in limited experimental studies, this protection was found to be lost in pathological conditions such as diabetes. These studies have been carried out in diabetic hearts which had similar (Badalzadeh et al. 2012) or decreased (Ren et al. 2011) tolerance to I/R injury. In the present study we found, for the first time to our knowledge, that IPost was not able to elicite a further protection although the isolated perfused hearts from diabetic rats had increased tolerance to I/R injury.

In contrast to clinical studies suggesting that the diabetic heart may have decreased tolerance to I/R injury (Zuanetti et al. 1993; Abbud et al. 1995), experimental findings indicate that the tolerance of diabetic hearts may not only be decreased but also similar and even increased (Feuvray and Lopaschuk 1997; Paulson 1997; Ravingerova et al. 2010,

Whittington et al. 2012). The reason for the discrepancy in the effects of diabetes on myocardial I/R injury is still ambiguous. Duration, severity and type of diabetes, kind of ischemia (i.e. no-flow or low-flow ischemia) and study protocols (*in vivo* or *in vitro*) are some of the proposed factors to explain the discrepancies in the sensitivity of diabetic hearts to I/R injury (Feuvray and Lopaschuk 1997; Paulson 1997; Whittington et al. 2012). It is suggested that hearts from STZ-diabetic rats have increased tolerance to I/R injury in the experimental conditions generally used acute diabetes (<4–6 weeks), a no-flow I/R protocol or glucose as the only substrate (Tosaki et al. 1996; Whittington et al. 2012). In the present work, we studied in the similar experimental conditions except the duration of diabetes and found that hearts isolated from 8-week diabetic rats had increased tolerance to ischemic injury as attested by an improvement in postischemic function and a significant decrease in TnI release. In consistent with our results, *in vivo* and *ex vivo* studies showed that hearts from rats or rabbits had increased tolerance to I/R injury in the chronic condition of type 1 diabetes (6–12 weeks) (Hadour et al. 1998; Ebel et al. 2003; Ooie et al. 2003; Ravingerova et al. 2003; Whittington et al. 2012). The decreased Ca^{2+} accumulation in the diabetic hearts by depressed activities of Na^{+}/H^{+} and Na^{+}/Ca^{2+} exchangers, the alterations in intracellular pH, a decreased rate of glycolysis, increased activation of protein kinase C and increased antioxidant capacity have been proposed as the possible mechanisms account for the increased tolerance of the diabetic hearts against I/R injury (Feuvray and Lopaschuk 1997; Paulson 1997; Moon et al. 1999; Chen et al. 2006).

From clinical point of view, our results showing the increased tolerance to I/R injury in diabetic hearts are inconsistent with data in clinical setting. As mentioned in the review of Ravingerova et al. (2010), possible factors lead to the differences between clinical and experimental studies may be the existence of other diseases including hypertension and hypercholesterolemia as well as antidiabetic drugs used in clinical conditions.

As a cardioprotective strategy against I/R injury, IPC or IPost have been shown to reduce infact size and arrhythmia and to elicite better recovery in cardiac contractile functions (Murry et al. 1986; Zhao et al. 2003; Sasaki et al. 2007; Pinheiro et al. 2009; Yin et al 2012; Buchholz et al. 2014). However, the presence of co-morbidities such as heart failure, hypertension, obesity and aging can significantly modify cardioprotective interventions (Snoeckx et al. 1993; Abete et al. 1996; Kristiansen et al. 2004). Diabetes is another co-morbidity which impairs the cardioprotection induced by IPost. Ren et al. (2011) found that the beneficial effect of this intervention has been found to be blunted in isolated perfused hearts from STZ-diabetic rats which had decreased tolerance to I/R injury. In addition, the loss of cardioprotective effect of IPost was also reported in hearts from type-1 or type-2 diabetic rodents which had similar tolerance to I/R injury (Przylenk et al. 2011; Badalzadeh et al. 2012). In agreement with these results, we found that IPost could not produce a protection even though hearts from STZ-diabetic rats had increased tolerance to I/R injury. Taken together it can be suggested that IPost seems to be a 'healthy heart phenomen'.

Conflict of interest. There is no conflict of interest.

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