Diabetes Blockade of Sevoflurane Postconditioning Is Not Restored by Insulin in the Rat Heart

Phosphorylated Signal Transducer and Activator of Transcription 3– and Phosphatidylinositol 3-Kinase–mediated Inhibition

Benjamin Drenger, M.D.,* Israel A. Ostrovsky, M.D.,** Michal Barak, M.D.,‡
Yael Nechemia-Arbely, Ph.D.,§ Ehud Ziv, Ph.D.,‖ Jonathan H. Axelrod, Ph.D.#

ABSTRACT

Background: The possibility of restoring sevoflurane postconditioning (sevo-postC) cardioprotection in diabetic animals is uncertain. We hypothesized that attenuation of myocardial injury by sevo-postC might be hindered by inhibition of signal transducer and activator of transcription (STAT) 3–regulated activity of phosphatidylinositol 3-kinase (PI3K) in diabetic animals. To determine whether postC cardioprotection can be restored by normoglycemia, we treated rats with insulin.

Methods: Diabetic or nondiabetic rats were randomly subjected to 30-min ischemia/reperfusion, with ischemic postC or sevo-postC, with and without mitochondrial adenosine triphosphate–dependent potassium channel blocker 5-hydroxy decanoate sodium and PI3K antagonist wortmannin. The infarct area, phosphorylated STAT3, and apoptosis were examined. Studies were repeated after insulin treatment.

Results: Ischemic postC and sevo-postC significantly reduced infarct size by 50% in the nondiabetic rats ($P < 0.002$), a phenomenon completely reversed by 5-hydroxy decanoate sodium and wortmannin. Diabetes mellitus blocked the protective effect of postC, and insulin treatment to achieve normoglycemia did not restore cardioprotection. Phosphorylated STAT3 nuclear retention was significantly increased after ischemia-reperfusion and was further enhanced in response to ischemic postC ($P < 0.05$) but was significantly reduced in diabetic rats (by 43%; $P < 0.01$).

Conclusions: The effective reduction in infarct size and apoptosis in the nondiabetic rat heart by postC was completely abrogated in diabetic rats. This inhibition is not relieved by insulin-induced normoglycemia. The PI3K pathway and mitochondrial adenosine triphosphate–dependent potassium channel activation are involved in the mechanism of postC. In diabetic rats, STAT3 activation was strongly reduced, as was postC cardioprotection, suggesting that the inability of insulin to restore postC may be attributed to diabetes-induced STAT3-mediated inhibition of PI3K signaling.

What We Already Know about This Topic

- Diabetes and acute hyperglycemia have attenuated cardioprotective signaling, in part, by impairing nitric oxide bioavailability and by attenuating the activation of mitochondrial adenosine triphosphate–dependent potassium channels.
- Postconditioning with ischemia or volatile anesthetics and insulin alone has produced cardioprotection by stimulating phosphatidylinositol 3-kinase; however, the efficacy of insulin for restoring postconditioning during diabetes is unknown.

What This Article Tells Us That Is New

- Treatment of diabetic rats with insulin to control blood glucose concentrations failed to restore protection against ischemia-reperfusion injury, elicited by sevoflurane or ischemic postconditioning; and these actions appeared to be related to the loss of signal transducer and activator of transcription 3 activation in diabetic myocardium.
- Alternative strategies to insulin therapy may be required to protect the diabetic heart against reperfusion injury.

* Associate Professor of Anesthesia, Department of Anesthesiology and Critical Care Medicine, Hadassah-Hebrew University Medical Center, Jerusalem, Israel. Current position: Visiting Professor, New York University Medical Center, New York, New York. ** Attending Anesthesiologist, Department of Anesthesiology and Critical Care Medicine, § Graduate Student, Goldyne Savad Institute of Gene Therapy, ‖ Associate Professor of Biochemistry, Diabetes Research Center, # Senior Research Associate, Goldyne Savad Institute of Gene Therapy, Hadassah-Hebrew University Medical Center. ‡ Lecturer in Anesthesiology, Department of Anesthesiology, Rambam Health Care Campus, Haifa, Israel.

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Address correspondence to Dr. Drenger: Department of Anesthesiology and Critical Care Medicine, Hadassah-Hebrew University Medical Center, PO Box 12000, Jerusalem, Israel 91120. drenger@cc.huji.ac.il. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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DIABETES mellitus is a major causal factor in the pathogenesis of coronary heart disease and congestive heart failure.[1,2] Perturbations in cardiac energy metabolism and insulin resistance are early events induced by the diabetic state.[3,4] During myocardial ischemia-reperfusion (I/R), the diabetic state interferes with the intrinsic protective–adaptive mechanism of myocardial ischemic preconditioning and postconditioning (postC), thus contributing to expanded infarct size and apoptosis.[5–8] Ischemic postC (isch-postC) mimics the protective effect of ischemic preconditioning; thus, its predictable therapeutic potential is applied at reperfusion and does not require anticipation of ischemia.[9] The administration of volatile anesthetics, such as isoflurane and sevoflurane, before or after the ischemic interval produces a similar pharmacological cardioprotection.[10–12] The volatile anesthetics exert their protective role by preserving mitochondrial oxidative mechanisms, among them mitochondrial adenosine triphosphate–dependent potassium (mK_{ATP}) channels and prosurvival proteins (*i.e.*, phosphatidylinositol 3-kinase [PI3K]/serine/threonine protein kinase [Akt]).[12,13]

In animal models, exogenous hyperglycemia, produced by glucose administration, and experimental induction of diabetes have both blocked the cardioprotective effects of ischemic preconditioning and postC, a phenomenon attributed to inhibition of the endothelial nitric oxide synthase system[14,15] and opening of mitochondrial permeability transition pores.[16] Although the inhibitory effects of hyperglycemia on preconditioning and postC could be nonuniformly reversed,[14–17] the effect of the diabetic state on both ischemic preconditioning and postC was irreversible.[7,18,19] However, the exact etiology for this disparity, and whether insulin administration might be beneficial, is not known. The unresponsiveness of the diabetic myocardium to insulin has been linked to a defect in insulin receptor substrate-1–associated PI3K activity and tyrosine phosphorylation of signal transducer and activator of transcription 3 (p-STAT3); these proteins regulate cell growth and survival.[20] STAT3 activation is required for initiation of PI3K/Akt signaling[21]; furthermore, insulin activation of PI3K signaling is also STAT3 mediated, making it critical for insulin signal transduction.[22]

The aims of the current study are to elucidate the role of mK_{ATP} channels and PI3K in the mechanisms of diabetic abrogation of isch-postC compared with sevoflurane postC (sevo-postC) cardioprotection and to examine whether normalization of hyperglycemia by insulin would restore postC. We hypothesize that in the diabetic rat heart, depression of postC cardioprotection might involve inhibition of the PI3K/Akt survival pathway *via* inhibition of STAT3 activation and that such inhibition also affects insulin's ability to restore cardioprotection. We demonstrate that sevo-postC promotes STAT3 phosphorylation. However, in diabetic rats, STAT3 activation is depressed and insulin therapy was ineffective in restoring the cardioprotective effects of postC.

Materials and Methods

All experiments conformed to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 85–23, revised 1996) and were approved by the Hebrew University School of Medicine Animal Care and Use Committee, Jerusalem, Israel.

Animal Preparation

Diabetes was induced in 3-month-old male Sprague–Dawley rats (weight, 310–340 g) by a single intravenous injection of 65 mg/kg streptozotocin (Sigma Aldrich, St Louis, MO) dissolved in 0.1 M citrate buffer (pH, 4.0) to the tail vein. Animals that maintained high blood glucose concentrations (greater than 150 mg/dl) on the second postinjection day, while fasting, and maintained blood glucose concentrations greater than 300 mg/dl in repeated blood tests (twice a week) during the fed state were declared as diabetic. The blood glucose concentration was determined using a glucose meter (Accutrend; Roche Diagnostics, Basel, Switzerland). Age-matched control animals received sham injections of the citrate buffer. All animals were maintained on the same laboratory animal diet (Teklad) with free water access. Four animals that developed ketoacidosis or weight loss greater than 40% were excluded. The experiments on the diabetic rats were performed 4 to 5 weeks after the streptozotocin injection. Rats in the insulin groups received 3 units per day of the intermediate-acting insulin (NPH, Humulin N; Lilly Pharma, Giessen, Germany) given during the 48 h before the experiment and 2 units of regular insulin 1 h before the experiment. *Normoglycemia* (defined as lower than 135 mg/dl) was verified twice daily and at the time of the experiment. General anesthesia was induced with intraperitoneal ketamine (10 mg/100 g body weight) and xylazine (0.3 mg/100 g body weight) (Sigma–Aldrich, Inc.), and the trachea was intubated with a 17-gauge polyethylene cannula under transillumination-supported direct vision. Mechanical ventilation was achieved with a positive-pressure respirator for small animals (model 683; Harvard Apparatus, South Natick, MA) using tidal volumes of 5 ml, 100% oxygen, and a rate of 50 breaths/min. Heart rate was monitored continuously with a tachograph preamplifier (13–4615-65; Gould Electronics, Inc., Eastlake, OH). Body temperature was maintained at 37°C using a heating lamp. Once heart rate stabilized, the heart was exposed through a left thoracotomy; and a left coronary ligation (30-min ischemia and 3-h reperfusion) was performed immediately at the upper third of the artery, just below the left atrial appendage. The extent of the regional myocardial ischemia was verified under direct vision with the appearance of epicardial cyanosis and changes in electrocardiographic tracing.

Experimental Protocols

The animals were randomly assigned to one of 23 experimental groups, as shown in figure 1. Two methods of cardioprotection (n = 8) were compared: A, Isch-postC, con-

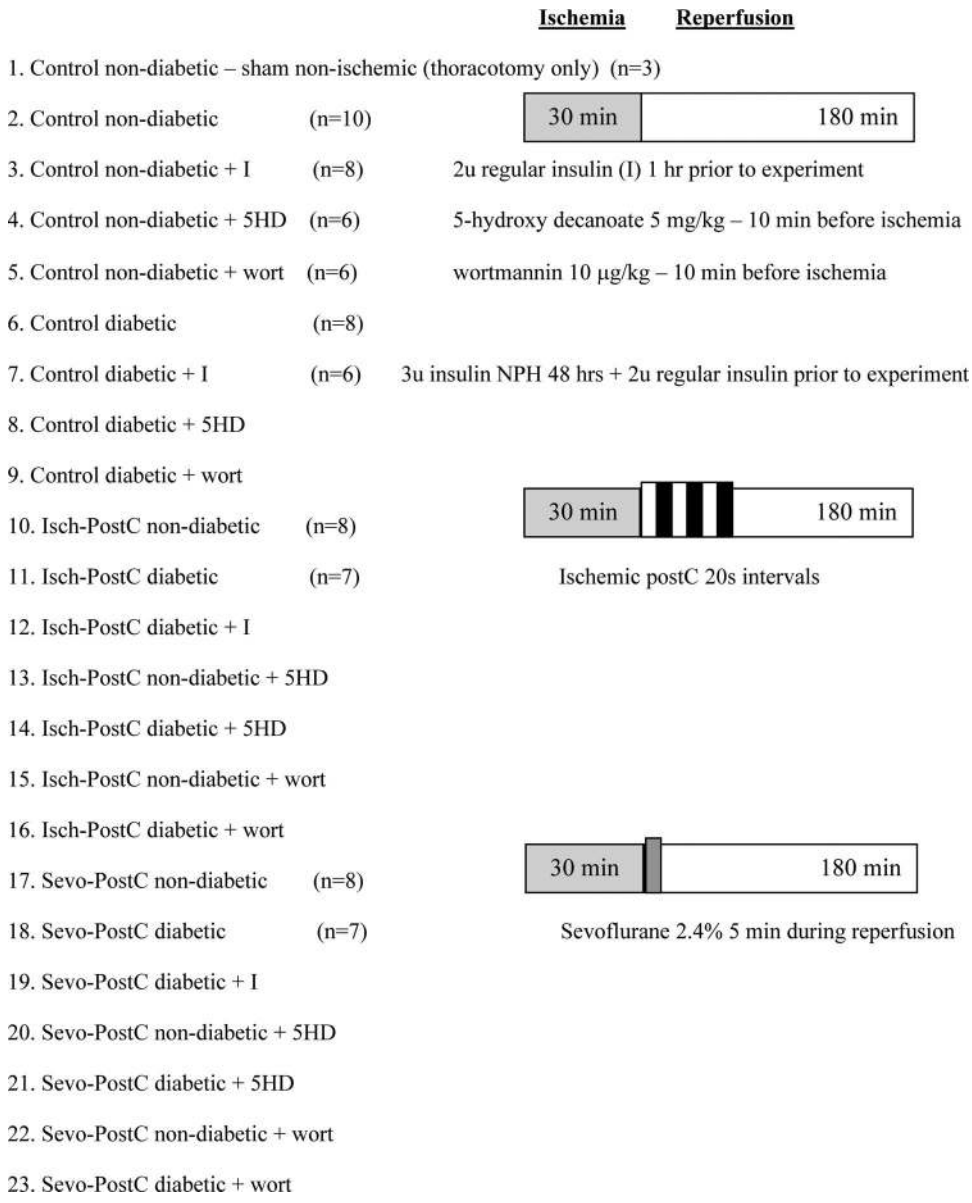

Fig. 1. Experimental groups with their respective protocols. The number of experiments is presented in parenthesis and is representative of all identical experiments. Isch-postC = ischemic postconditioning; sevo-postC = sevoflurane postconditioning.

sisting of three 20-s intervals of occlusion/reperfusion on the initiation of the reperfusion period; B, Sevo-postC, sevoflurane, 2.4% (one minimal alveolar concentration equivalent), given by inhalation for 5 min *via* sevoflurane vaporizer (Sevotec 5; Datex-Ohmeda, Tewksbury, MA) connected inline, immediately on initiation of reperfusion. The expiratory sevoflurane concentration was measured at the end of the endotracheal cannula (Datex Capdiocap II, Helsinki, Finland).

The interventions were performed in the healthy and diabetic rats, which were divided into two groups: A, untreated diabetes (n = 8), rats with hyperglycemia greater than 300 mg/dl; and B, treated diabetes (n = 6). The experiments on the untreated diabetic rats were conducted in the absence and presence of 5-hydroxy decanoate sodium (5-HD) (5 mg/kg; Sigma Aldrich) as a specific mK_{ATP} blocker; and of wortmannin (10 μg/kg; Sigma Aldrich), the specific PI3K inhibitor. The experiments were repeated twice: the first set was used to calculate infarct size proportionate to the area at risk by using triphenyltetrazolium chloride staining (Sigma Aldrich), and the second set was fixed in 4% buffered formaldehyde and embedded in paraffin for terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) and STAT3 staining.

Determination of Infarct Size

At the conclusion of the experiment, the left coronary artery was reoccluded and the aortic root was reperfused with 5–10 ml Evans blue dye, 0.2%, in dextran, 1%, which stained the normal tissue. The heart was then frozen at −20°C before

Table 1. Characteristics of Control and Diabetic Rats

Characteristics	Nondiabetic Rats			Diabetic Rats			Diabetic Rats Treated with Insulin		
	Control I/R (n = 26)	Isch-PostC (n = 24)	Sevo-PostC (n = 24)	Control I/R (n = 22)	Isch-PostC (n = 22)	Sevo-PostC (n = 21)	Control I/R (n = 6)	Isch-PostC (n = 6)	Sevo-PostC (n = 6)
Body weight, g	334 ± 9	332 ± 11	338 ± 7	211 ± 12	218 ± 21	228 ± 11	227 ± 12	230 ± 11	229 ± 14
Initial weight, g				330 ± 12	333 ± 13	339 ± 15	348 ± 11	353 ± 9	353 ± 11
% Change*				36.1	34.5	32.7	34.7	34.8	35.1
Heart weight, g†	1.23 ± 0.07	1.24 ± 0.08	1.30 ± 0.08	1.25 ± 0.05	1.31 ± 0.1	1.30 ± 0.1	1.33 ± 0.07	1.32 ± 0.1	1.29 ± 0.11
Heart/Body weight ratio	0.37	0.37	0.38	0.59	0.60	0.57	0.58	0.57	0.56
Heart rate (at 2-min Reperfusion)†	322 ± 12	313 ± 14	302 ± 9	308 ± 9	312 ± 11	306 ± 12	326 ± 15	319 ± 10	312 ± 308
Animals excluded									
>40% Weight loss					4			1	
Ketoacidosis					1				
Faulty staining				1	1	1			
Animals that died									
Ischemia	2					1			
Reperfusion	2	1		2					
Diabetes induction					3			1	
Blood glucose DOS, mg%	77 ± 8	75 ± 8	79 ± 7	503 ± 81	580 ± 90	585 ± 18	121 ± 7	126 ± 10	131 ± 9

* $P = 0.002$ *vs.* nondiabetic rats; † P = nonsignificant.

DOS = day of surgery; I/R = ischemia-reperfusion; Isch-postC = ischemic postconditioning; Sevo-postC = sevoflurane postconditioning.

slicing into 10 transverse slices (thickness, 1 mm). The slices were incubated (37°C) for 15 min in buffered triphenyltetrazolium chloride, 1%, adjusted to pH 7.4, and then incubated for 4 h in formaldehyde, 4%. The viable area at risk was stained red while the infarcted area remained unstained. The area at risk and the infarcted area were determined by planimetry using digital photography and a custom-built photographic apparatus, and the ratio of pixels of the areas was calculated. Determinations of risk zone size and area of infarction were performed by a blinded investigator.

Detection of Myocardial Apoptosis

Apoptosis was assessed through a TUNEL assay (n = 8 for each group). The apoptotic cells were identified in formalin-fixed paraffin-embedded heart tissue sections using an *in situ* cell death detection kit (POD; Roche Diagnostics Corp, Indianapolis, IN), according to the manufacturer's protocol using a fluorescence microscope. Apoptosis quantification was performed by counting TUNEL-positive cells per high-power field (magnification, ×400). A total of 10 fields per heart were analyzed, and the mean ± SD was determined.

Immunohistochemical Analysis of STAT3

Samples of 4-μm slices of myocardial tissue embedded in paraffin (n = 8 for each group) were stained by p-STAT3 using monoclonal rabbit anti-mouse p-STAT3 (tyrosine 705) (Cell Signaling Technology, Inc., Danvers, MA), diluted 1:500, followed by biotinylated goat anti-rabbit (Jackson ImmunoResearch Laboratories, West Grove, PA), diluted 1:5,000, amplified using a tyramide signal amplification kit (PerkinElmer, Waltham, MA) and developed with 3-amino-9-ethylcarbazole (DAKO, Carpenteria, CA).[23] The proportion of positive p-STAT3 nuclei per high-power field was measured using an automated image analysis system (Ariol SL-50; Applied Imaging, Grand Rapids, MI).

Statistical Analysis

Data are reported as mean ± SD. Infarct size and area at risk were calculated and compared for various procedures with one-way ANOVA. Comparisons between the diabetic and healthy animals for particular experimental factors were analyzed with two-tailed *t* tests, with corrections for multiple comparisons (Tukey–Kramer multiple comparison test). $P < 0.05$ was considered statistically significant. All analyses were performed using SAS statistical software (SAS Institute Inc., Cary, NC).

Results

One hundred sixty-three rats were tested in the triphenyltetrazolium chloride–stained area-of-infarct experiments, and an additional 48 animal formalin-fixed paraffin-embedded heart tissue sections were collected for TUNEL and STAT3 staining. Table 1 delineates general characteristics of the animals studied and those that were excluded or died. Baseline weight was similar in all groups; however, at the time of the experiment, the diabetic animals displayed weight loss of approximately 35% compared with control animals ($P = 0.002$), with unchanged heart weight. The blood glucose concentration on the day of the experiment in normal control rats was 77 ± 7 mg/dl compared with 503 ± 81 mg/dl in the diabetic rats and 121 ± 7 mg/dl in those receiving insulin treatment (table 1). The heart rate was not different among groups before and during the ischemic period, except for a slight decrease during the short period of sevoflurane administration at reperfusion ($P = 0.64$) (table 1).

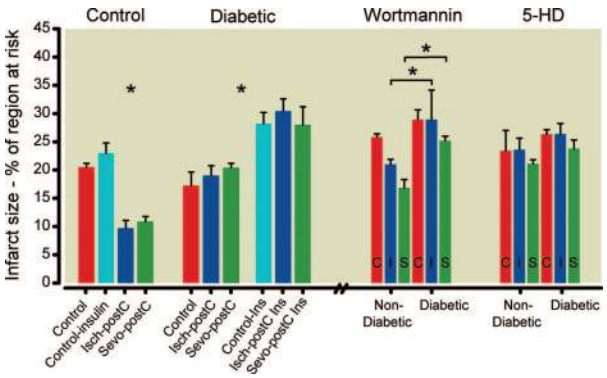

Fig. 2. Area of infarct as percentage of the ischemic region at risk by triphenyltetrazolium chloride staining. Bars indicate the mean ± SD of the following sets of experiments: control (C) ischemia-reperfusion (I/R), ischemic postC (isch-postC; I), sevoflurane postC (sevo-postC; S), 5-hydroxy decanoate sodium (5-HD) as a mitochondrial adenosine triphosphate–dependent potassium channel antagonist, and wortmannin as a phosphatidylinositol 3-kinase antagonist. Each was given to nondiabetic and diabetic animals. Insulin (Ins) experiments were performed after 48-h treatment in the diabetic animals. *$P < 0.002$ (control experiments: isch- and sevo-postC *vs.* control I/R). *$P < 0.05$ (diabetic experiments with Ins [control and isch-postC] *vs.* diabetic without Ins and diabetic sevo-postC with and without Ins). *$P = 0.028$ for wortmannin action on isch- and sevo-postC in the diabetic group.

Sevo-postC and isch-postC similarly reduced infarct size proportionate to the area at risk to 11% and 9%, respectively, compared with 20% in control ischemic animals ($P = 0.002$; fig. 2). This protective effect was nullified in the diabetic animals and was not restored in the presence of insulin therapy. Furthermore, in the presence of insulin therapy, infarct size in the treated animals increased significantly in all the diabetic groups and was refractory to sevo- and isch-postC protection (22–30%; $P < 0.05$ in diabetic control and in both postC groups, compared with the reciprocal baseline value). Insulin treatment in the nondiabetic rats did not affect infarct size.

Both 5-HD, the mK_{ATP} channel antagonist, and wortmannin, the PI3K antagonist, completely reversed the protective effect of sevo- and isch-postC (fig. 2). Furthermore, wortmannin significantly increased the infarct size in both postC groups in the diabetic hearts ($P = 0.028$). On the other hand, 5-HD administration did not change infarct size in the diabetic hearts.

Quantification of TUNEL-positive myocyte nuclei (fig. 3) revealed significantly more apoptotic cells in control ischemic animals compared with sevo- or isch-postC groups (13.7 ± 4.7% *vs.* 9.1 ± 4.6% and 8.2 ± 4.1%, respectively; $P = 0.05$ and $P = 0.039$, respectively). In the diabetic heart samples, many apoptotic cells were also observed (13.5 ± 3.4%), but the proportion was not greater than in control ischemic samples.

Immunostaining for p-STAT3 showed strong activation in response to I/R stimulus; the amount of p-STAT3–positive nuclei (in 10 high-power fields; magnification, ×400) was 8.6 ± 9.1 *versus* 86.43 ± 48.79 (sham-treated *vs.* control ischemic hearts; $P < 0.05$) (fig. 4). p-STAT3 activation was further increased in response to isch-postC (112.2 ± 82.7, $P < 0.05$), which was not apparent with sevo-postC stimulation (91.9 ± 68.2). However, in the diabetic heart, a significant decrease in p-STAT3 activation was observed in all three groups (52.4 ± 41.5, $P = 0.014$). The decrease in nuclear staining in diabetic rats was particularly prominent in areas not at risk, especially the right ventricle and septum (fig. 5). Thus, ischemia-induced p-STAT3 activation correlated with protection from ischemic injury and was significantly decreased in the isch-postC–resistant diabetic heart.

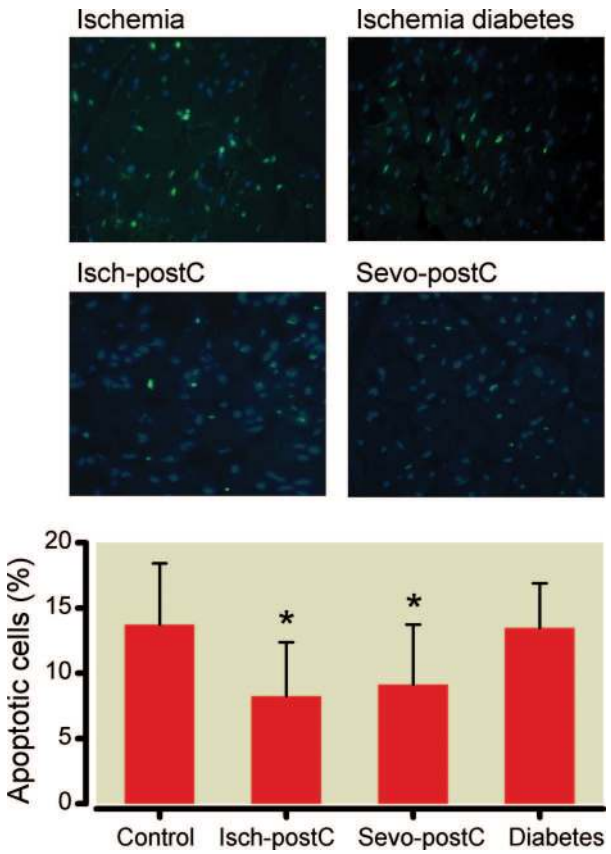

Fig. 3. Myocardial terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) staining (n = 8 for each group). Representative high-power field with TUNEL-stained apoptotic nuclei in green and non-apoptotic nuclei in blue. Apoptosis quantification was performed by counting TUNEL-positive cells (magnification, ×400). Data are given as mean ± SD percentage of apoptotic cells (10 fields per heart were analyzed). *$P = 0.05$ and *$P = 0.039$. Isch-postC = ischemic postconditioning; sevo-postC = sevoflurane postconditioning.

Discussion

This study demonstrates that the effectiveness of myocardial protection by sevo-postC is hindered by diabetes in the rat heart. Furthermore, we show, for the first time to our knowl-

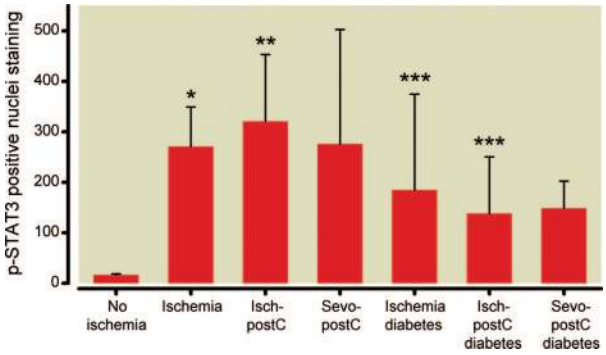

Fig. 4. Mean count of phosphorylated signal transducer and activator of transcription 3 (p-STAT3)–positive nuclei staining (mean count in 10 high-power fields; magnification, ×400) in each of the study groups (n = 8 for each group), with and without diabetes. Nonischemic experiments were compared with sham-operated animals without an ischemic interval (n = 3). *$P < 0.05$. A significant additional increase in p-STAT3 was observed in the ischemic postconditioning (isch-postC) group compared with the ischemia-reperfusion group (**$P = 0.05$). The change in sevoflurane postconditioning (sevo-postC) was smaller ($P = 0.09$). Diabetes significantly reduced p-STAT3 activity (***$P = 0.014$).

edge, that correction of the hyperglycemic state by insulin treatment exacerbates, rather than prevents, the deleterious effect of diabetes on ischemic injury and is refractory to postC protection. We also demonstrated the important roles of PI3K/Akt and mK_{ATP} channels in mediating postC cardioprotection. The inhibitory effects of diabetes on this PI3K/Akt salvage pathway were resistant to insulin, an effect that might be attributed to the decrease we observed in diabetes-mediated tyrosine phosphorylation of STAT3.

The Janus kinase (JAK) 1–STAT pathway consists of cytokine-sensitive signaling molecules that are activated by stress conditions (*e.g.*, myocardial ischemia, mechanical stress, or inflammation)[24] and have been implicated in cardioprotection by prevention of apoptosis, cardiac hypertrophy, and inflammation,[22] and postC.[25] STAT3 activation by brief episodes of I/R underlies the transcriptional up-regulation of the inducible nitric oxide synthase gene expression. This may trigger cardioprotection in the form of late preconditioning[26] and is a required step in the signal transduction pathway of PI3K, which is known to enhance postC-mediated reduction in infarct size.[21]

The role of JAK–STAT in diabetic myocardium has not been fully characterized. Diabetes seems to promote defects in PI3K/Akt signaling[20] and reduce STAT3 phosphorylation independently of Akt.[27] We describe two interesting observations in the diabetic heart: (1) p-STAT3 nuclear activation is significantly attenuated in diabetes and (2) administration of the PI3K antagonist, wortmannin, in diabetes aggravates infarct size during postC, indicating that PI3K activation is specifically blocked in those with diabetes. Because p-STAT3 inhibition and PI3K inhibition were linked pathways in the cardiac myocyte,[21] in our study, we propose that the defect in diabetes-mediated PI3K/Akt signaling is attrib-

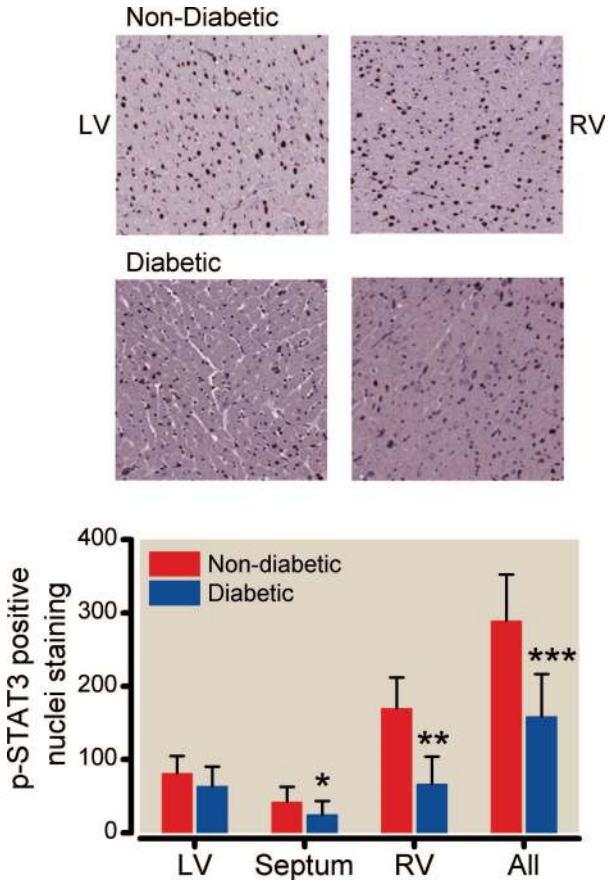

Fig. 5. Representative high-power field phosphorylated signal transducer and activator of transcription 3 (p-STAT3) stained nuclei in red. Mean count of phosphorylated p-STAT3 nuclei staining in the different sections of the heart in response to ischemia-reperfusion stimulus is presented in nondiabetic and diabetic animals (***$P < 0.005$). Nonischemic ventricular segments are mainly affected by diabetes. *$P < 0.05$ and **$P < 0.0001$. LV = left ventricle; RV = right ventricle.

uted, in part, to depressed p-STAT3. Furthermore, the I/R-induced short-term increase in p-STAT3 activation was particularly evident in the normal myocardium not directly exposed to ischemia, thus emphasizing that the heart, as a whole, spurs its defensive mechanism. The fact that postC is abrogated in the diabetic state emphasizes the important role of the JAK–STAT system on PI3K. Although we cannot prove direct causality between reduction in p-STAT3 and failure to induce postC cardioprotection in the diabetic heart, our data confirm an increase in p-STAT3 during sevo-postC, an effect again abrogated by diabetes. Our data also confirm that both isch- and sevo-postC significantly attenuate apoptosis in our *in vivo* model, findings that are in agreement with a similar observation in an isolated guinea pig heart model of reduction in apoptosis by sevoflurane.[8]

The current study demonstrates that wortmannin administration in diabetes resulted in a larger infarct size. These findings are in agreement with a previous study[28] that showed an excessive apoptotic injury caused by wortmannin during reoxygenation in diabetic rat myocytes; however, the

mechanism was not completely understood. In another model of hemopoietic progenitor cells, Minshall *et al.*[29] showed that PI3K is necessary to prevent apoptosis and that wortmannin-induced augmented apoptosis might be attributed to PI3K parallel inhibition of several intracellular alternative pathways, which use receptors with intrinsic kinase activity. We suggest that, in the current study, diabetes magnified the inhibitory effect of wortmannin in a yet unexplained manner; however, because it may affect tyrosine kinase activity,[28] pertinence to insulin resistance might be possible.

Huisamen[30] showed that the inability of insulin to activate Akt was attributed to faulty transduction of signals in the diabetic myocardium. By using wortmannin, they abolished insulin-stimulated glucose uptake and Akt activation, thus suggesting that the signaling pathways converge at activation of PI3K. In our study, the blockade of postC and the increased response of wortmannin in diabetes emphasize that inhibition of PI3K/Akt is a crucial insult in diabetes. Although not directly shown, the inability of insulin to restore postC in the diabetic animal is in line with its inability to activate the PI3K/Akt pathway. Our findings of blunted STAT3 activation in the diabetic myocardium might explain the resistance of the heart to insulin[20] and the inability of the diabetic heart to respond to both isch-postC and sevo-postC protection. They might also explain the inability of insulin to reverse the abrogation of postC protection.

Several studies[6,31,32] indicated that, under diabetic conditions, mitochondrial sites, such as mK_{ATP} channels and mitochondrial permeability transition pores, are altering their state during the first few minutes of reperfusion. These mitochondrial changes may mediate cardiomyocyte death. Indeed, in the current study, the use of 5-HD, a specific antagonist of the mK_{ATP} channels, was effective in reversing postC in the nondiabetic myocardium but did not have any effect on the diabetic myocardium. These findings support the hypothesis that mK_{ATP} channels become inactive and fail to elicit postC cardioprotection in the diabetic myocardium.

Interestingly, studies on simulated diabetes, using the model of exogenously induced hyperglycemia, were able to reverse the abrogating action of hyperglycemia on postC. In hyperglycemic animals, the cardioprotective effect of anesthetic postC could be restored by up-regulation of endothelial nitric oxide synthase[15] or inhibition of the mitochondrial permeability transition pores.[16] Those exact mechanisms were irresponsive in diabetes, presumably because of changes in mitochondrial membrane potentials that were leading to a certain degree of mitochondrial uncoupling.[7,18,33] An additional signaling enzyme affected in diabetes is glycogen synthase kinase-3β. Its activation in diabetes alters the balance of important cellular pathways of cardioprotection, including tyrosine kinase, JAK–STAT, and PI3K.[19] The role of the diabetic activation of glycogen synthase kinase-3β was recently elucidated, revealing significant insult to cardiac energy metabolism, with lipid accumulation, inflammation, and remodeling.[34] We speculate that inhibition of STAT3 and PI3K expression in diabetes, as observed in the current study, may lead to glycogen synthase kinase-3β activation, subsequently altering mitochondrial membrane potentials, with abrogation of postC cardioprotection. We suggest that the diabetic suppression is multifactorial, and the previously described cellular and mitochondrial sites might all be involved in revoking the postC state. Our current research model is not sufficient to confirm all these hypotheses, and additional studies are needed to explain the inability of preconditioning and postC to protect the diabetic rat heart.

Our attempt to restore postC cardioprotection by normalizing blood glucose concentrations with 48 h of insulin therapy in those diabetic rats was unfruitful. Prolonged hyperglycemia promotes a glycation reaction, a nonenzymatic reaction of various reducing sugars that leads to cell injury and the accumulation of advanced glycation end products. These advanced glycation end products are important in the development of oxidative stress, diabetic complications, and insulin resistance.[35] Prompt insulin administration may ameliorate those changes; although not tested in the current study, a longer period of insulin therapy might have a different effect on restoring postC.

Interestingly, insulin administration before the I/R event in our model of diabetic animals resulted in a phenomenon of a larger infarct size than without insulin therapy. This phenomenon is difficult to explain. It is possible that insulin as an anabolic hormone may increase cellular metabolism in the ischemic region already exposed to a high rate of lipid and glucose oxidation.[36–38] Because the ischemia is of a no-flow type, the heart is in a state of pressure overload and high myocardial oxygen consumption, explained by the high wall stress and paradoxic regional systolic bulging in the uninvolved myocardial segments.[39,40] This explanation should be interpreted with caution because myocardial oxygen consumption was not specifically measured in the current study.

On occasions when insulin is given as part of a metabolic cocktail, composed of glucose, insulin, and potassium, it reduced infarct size in animals[28]; however, the timing of applying this intervention is controversial. In a recent human study,[41] pretreatment with glucose, insulin, and potassium reduced the severity of the stress-induced contractile dysfunction and improved postischemic reperfusion; however, another study[28] suggested that insulin effectively attenuates I/R-induced apoptosis when given after the ischemic interval (during reperfusion). According to our findings, we suggest that caution should be taken not to add insulin before the planned ischemic period.

We conclude that diabetes abrogates postC by the defects it creates in insulin-mediated PI3K/Akt signaling and by inhibiting STAT3 activation. Still, offering anesthetic postC as a cardioprotective technique in diabetic patients to attenuate the foreseen ischemic insult is an important therapeutic target. Further attempts are justified to elucidate the crucial

cellular sites to be targeted for making postC an effective intervention for myocardial protection in the diabetic state.

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