The Beneficial Effects of Ranolazine on Cardiac Function After Myocardial Infarction Are Greater in Diabetic Than in Nondiabetic Rats

Iordanis Mourouzis, Polixeni Mantzouratou, Georgios Galanopoulos, Erietta Kostakou, Arvinder K. Dhalla, Luiz Belardinelli and Constantinos Pantos

J CARDIOVASC PHARMACOL THER 2014 19: 457 originally published online 19 March 2014
DOI: 10.1177/1074248414524481

Journal of Cardiovascular Pharmacology and Therapeutics
2014, Vol. 19(5) 457-469

DOI: 10.1177/1074248414524481
cpt.sagepub.com
SAGE

The Beneficial Effects of Ranolazine on Cardiac Function After Myocardial Infarction Are Greater in Diabetic Than in Nondiabetic Rats

Iordanis Mourouzis, MD[1], Polixeni Mantzouratou, MD[1], Georgios Galanopoulos, MD[1], Erietta Kostakou, BSc[1], Arvinder K. Dhalla, PhD[2], Luiz Belardinelli, MD[2], and Constantinos Pantos, MD[1]

Abstract

Ranolazine (RAN) is known to exert both anti-ischemic and antidiabetic actions. Thus, this study has explored the hypothesis that RAN would have greater effect on the recovery of cardiac function in diabetic mellitus (DM) rat hearts following myocardial infarction (MI). Myocardial infarction was induced in nondiabetic (MI, n = 14) and diabetic (streptozotocin induced; DM-MI, n = 13) Wistar rats by permanent ligation of the left coronary artery. Cardiac function was evaluated using echocardiography (left ventricular ejection fraction %) and in isolated heart preparations by measuring left ventricular developed pressure (LVDP), and the positive and negative first derivative of LVDP ($\pm$ dp/dt). Ranolazine (20 mg/kg, ip once a day) was administered 24 hours after surgical procedure for 4 weeks to nondiabetic (MI + RAN, n = 17) and diabetic rats (DM-MI + RAN, n = 15). The RAN improved the recovery of function in both the nondiabetic and the diabetic postinfarcted hearts but this effect was greater and achieved statistical significance only in the diabetic group. The RAN resulted in increased levels of phosphorylated protein kinase B (Akt) and mammalian target of rapamycin (mTOR, a component of Akt signaling) in both nondiabetic and diabetic infarcted hearts without changes in the activation of mitogen-activated protein kinases (MAPKs; p38 MAPK, c-Jun N-terminal kinase, and extracellular signal-regulated kinase). In addition, in diabetic hearts, RAN resulted in a significant increase in the ratio of sarcoplasmic Ca^{2+}-ATPase/phospholamban (a target of Akt signaling, 2.0-fold increase) and increased levels of phosphorylated calcium-regulated adenosine monophosphate-activated protein kinase (AMPK; 2.0-fold increase). In diabetic animals, RAN increased insulin and lowered glucose levels in serum. In conclusion, the beneficial effect of RAN on the recovery of cardiac function after MI was greater in DM rats. This response was associated with activation of Akt/mTOR and AMPK. These findings provide a plausible explanation for the results of the Type 2 Diabetes Evaluation of Ranolazine in Subjects With Chronic Stable Angina (TERISA) trial, which showed a greater antianginal effect of RAN in patients with coronary artery disease and diabetes.

Keywords

ranolazine, myocardial infarction, intracellular kinase signaling, diabetes, insulin

Introduction

Diabetes and coronary artery disease (CAD) are often present in the same patient.[1] Several studies have shown that diabetic patients are twice as likely to die after myocardial infarction (MI) compared to nondiabetic patients.[2,3] Furthermore, the incidence of heart failure is high in diabetic patients after MI despite primary angioplasty and current optimal treatment.[4]

Ranolazine (RAN) is a clinically used antianginal drug with anti-ischemic actions due to its beneficial effect on Na^+ and Ca^{2+} homeostasis upon stress.[5,6] Ranolazine, in addition to the anti-ischemic effects, has also been shown to have antiarrhythmic[7] and antidiabetic actions.[8,9] In the Metabolic Efficiency With Ranolazine for Less Ischemia in Non-ST-Elevation Acute Coronary Syndrome-Thrombolysis in Myocardial Infarction 36 (MERLIN TIMI-36) trial,[9] RAN was found to lower glycosylated hemoglobin A1c (HbA1c) in patients with ischemic heart disease and poorly controlled diabetes. Hence, in patients with ischemic heart disease, RAN has both anti-ischemic and antidiabetic effects. Consequently, these patients are likely to

[1] Department of Pharmacology, University of Athens, Athens, Greece
[2] Gilead Sciences Incorporated, Fremont, CA, USA

Manuscript submitted: October 16, 2013; **accepted:** January 22, 2014.

Corresponding Author:
Constantinos Pantos, Department of Pharmacology, University of Athens, 75 Mikras Asias Ave, 11527 Goudi, Athens, Greece.
Email: cpantos@med.uoa.gr

benefit the most from RAN treatment. Consistent with this interpretation, the results of the recently published Type 2 Diabetes Evaluation of Ranolazine in Subjects With Chronic Stable Angina (TERISA) trial showed that in patients with type 2 diabetes, established CAD and stable angina, RAN was more effective than placebo in reducing the primary outcome of average weekly angina episodes as well as average weekly sublingual nitroglycerin use.[10] Interestingly, the antianginal effect of RAN was found to be greater in patients with high HbA1c.[10] The basis for this greater antianginal effect of RAN in patients with poorly controlled diabetes is not understood. Thus, the present study was designed to compare the effects of RAN in diabetic and nondiabetic rats in an experimental model of myocardial infarction and to seek insights into the mechanisms of the difference in response to RAN. The modulatory effect of RAN on calcium homeostasis upon stress may be of physiological relevance. Calcium is shown to regulate prosurvival pathways, such as protein kinase B (Akt) and adenosine monophosphate-activated protein kinase (AMPK).[11-13] Protein kinase B is an important component of insulin signaling and can control cardiac function via its regulatory role on contractile proteins.[14,15] On the basis of this evidence, the present study explored potential changes in stress-induced cell signaling after RAN treatment. This issue has not been previously addressed.

Methods and Materials

Animals

Male Wistar rats, 300 to 360 g, were maintained on a 12-hour light–dark cycle. Handling of animals was performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health Guide (NIH Pub. No. 83-23, Revised 1996). University ethics review board approved this experimental protocol.

Induction of Diabetes

Streptozotocin (STZ; Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany) was diluted in 0.1 mol/L sodium citrate buffer (pH 4.5) and injected intraperitoneally (ip) at a single dose of 35 mg/kg in order to induce diabetes mellitus (DM) as described previously.[16] The STZ injection results in selective destruction of pancreatic β cells and in development of DM within 3 days. The dose of 35 mg/kg was selected because we previously showed that this dose can induce glucosuria without concomitant ketoacidosis.[16] In fact, initial experiments with doses of STZ as high as 60 mg/kg resulted in diabetes with significant ketonuria. Urine samples from STZ-treated animals were analyzed for glucose and ketone levels 4 days after the injection using Keto-Diastix (Bayer Hellas AG, Athens, Greece). Rats were subjected to coronary ligation 30 days after STZ injection.

Experimental Model of Myocardial Infarction

Surgical ligation of the left coronary artery was performed in order to generate myocardial infarction as previously described.[17,18] Initial anesthesia was achieved with injection of ketamine (70 mg/kg, ip) and midazolam (0.1 mg/kg, ip). Rats were subjected to intubation via a tracheal cannula and ventilated with a small rodent ventilator (Harvard Apparatus, Holliston, Massachusetts; Inspira, 50 breaths/min, 1 mL/100 g tidal volume). Inhaled sevoflurane at doses 1% to 2% was used to maintain anesthesia during surgery. A 6-0 silk suture was utilized for ligation of the left coronary artery. During the procedure, electrocardiogram recording was applied in order to monitor changes in heart rate and verify ST-segment changes typical of myocardial infarction. Body temperature was kept constant with a Harvard Homeothermic blanket. Mortality in nondiabetic animals was 18.4% (7 of 38 animals) while it increased up to 33.3% in diabetic animals (14 of 42 animals) during the first 24 hours following surgical procedure. This study included myocardial infarctions with a scar area of 70 to 130 mm^2, which corresponds to 30% to 50% of the left ventricle (LV). Sham-operated rats were subjected to the same procedure, but no ligation of the suture was performed.

Administration of RAN

Ranolazine was provided by Gilead Sciences, Inc (Fremont, California). A stock solution of RAN was prepared in distilled water with the addition of hydrochloride in order to adjust pH at 4.0. The solution was filtered using a 0.22-μm pore sterilizing membrane filter. From this stock solution (concentration 1 g/10 mL), a fresh solution was made every day by diluting 10 times in normal saline. Ranolazine was administered ip to all rats once a day. The dose of 20 mg/kg RAN was selected after a series of pilot pharmacokinetic studies. This dose resulted in a peak concentration of 8000 ng/mL of RAN and an average 24 hours concentration of approximately 1500 ng/mL in plasma which is clinically relevant.[19]

Experimental Protocol

Two groups of rats were studied, nondiabetic (n = 43) and diabetic rats (n = 40).

Nondiabetic rats were divided into the following groups: sham-operated rats (SHAM, n = 12); rats with myocardial infarction treated with vehicle (MI, n = 14); and rats with myocardial infarction treated with RAN 20 mg/kg (MI + RAN, n = 17).

Similarly, diabetic rats (n = 40) were divided into the following groups: sham-operated diabetic rats (DM-SHAM, n = 12), diabetic rats with myocardial infarction treated with vehicle (DM-MI, n = 13), and diabetic rats with myocardial infarction treated with RAN 20 mg/kg (DM-MI + RAN, n = 15).

Ranolazine (20 mg/kg) was administered ip once daily for 4 weeks starting 24 hours postsurgery, whereas SHAM, MI, DM-SHAM, and DM-MI groups received intraperitoneal injections of vehicle.

At 4 weeks postsurgery (18-20 hours after the last dose of RAN), anesthesia was induced with intraperitoneal injection of ketamine hydrochloride and all rats were subjected to

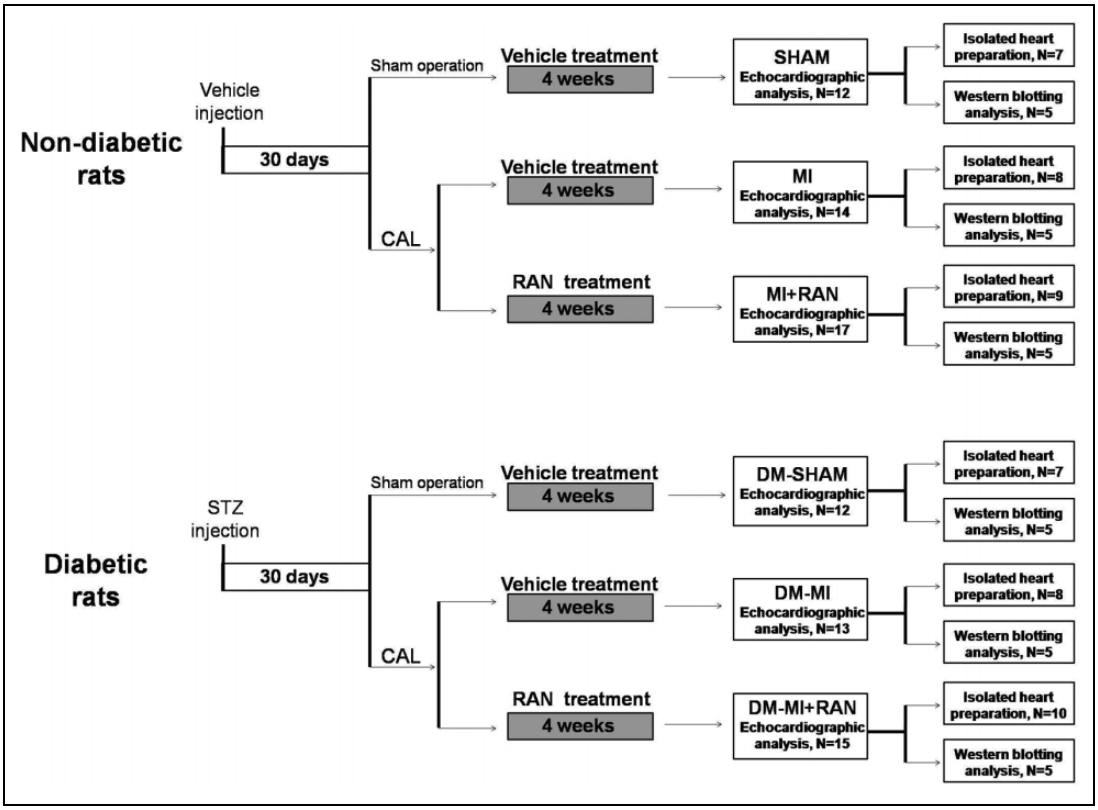

Figure 1. Schematic of the design of the experimental protocol. CAL indicates coronary artery ligation; STZ, streptozotocin; RAN, ranolazine.

echocardiographic examination for assessment of cardiac function. In addition, after randomization, a number of hearts from each group were also perfused according to Langendorff technique for assessment of cardiac function under isometric conditions (SHAM, n = 7; MI, n = 8; MI + RAN, n = 9; DM-SHAM, n = 7; DM-MI, n = 8; and DM-MI + RAN, n = 10; Figure 1).

Left ventricle was isolated from all hearts and scar tissue was separated from the viable left ventricular tissue. Weights of the scar tissue, the viable LV tissue, and the lungs were measured and recorded. The area of the scar tissue (in mm^2) was quantified.

The ratio of wet lung weight to body weight (LW/BW) was used to assess the incidence of pulmonary congestion after myocardial infarction. Mean value of LW/BW in SHAM group $\pm$ ($3 \times$ standard deviation) corresponding to the maximal 99% confidence interval was set as a cutoff point as described previously.[20]

Blood was collected from the right atrium and centrifuged in order to collect serum. Serum was stored at $-70^{\circ}C$ and used for glucose and insulin measurements.

Immunoblotting analysis was performed in the noninfarcted specimens of LV (n = 5 samples per group) from hearts that were not perfused in the Langendorff apparatus.

Echocardiography

At 4 weeks after ligation, anesthesia was performed with intraperitoneal injection of ketamine hydrochloride (100 mg/kg) and heart function was estimated using echocardiography as described previously.[17,18] Acquisition of echocardiographic images in short and long axes was achieved with a Vivid 7 version Pro ultrasound system (GE Healthcare, Wauwatosa, Wisconsin) equipped with a 14.0-MHz probe (i13L). Images were evaluated by 2 independent, experienced operators blinded to the experimental groups.

Estimated parameters include LV internal diameter at end-diastolic phase (LVIDd) and LV internal diameter at end-systolic phase (LVIDs), LV posterior wall thickness at end-diastolic phase, and the ejection fraction (EF). The Simpson equation was used for the calculation of EF. Systolic velocity of posterior wall (SVPW) radial displacement was determined and used to assess the regional contractile function of the LV myocardium.

Isolated Heart Preparation

Perfusion of isolated hearts at a constant flow was achieved according to the Langendorff technique.[21] Anesthesia was performed with intraperitoneal injection of ketamine hydrochloride (150 mg/kg) and heparin 1000 IU/kg was administered before thoracotomy. The hearts were excised and mounted on the aortic cannula within 60 seconds. Hearts were perfused with Krebs-Henseleit buffer (composition: sodium chloride 118 mmol/L, sodium bicarbonate 25 mmol/L, potassium phosphate monobasic 1.2 mmol/L, potassium chloride 4.7 mmol/L, magnesium sulfate 1.2 mmol/L, calcium chloride 1.4 mmol/L, and glucose 11 mmol/L). Pacing was accomplished with a

Harvard pacemaker at 320 beats/min. A latex balloon filled with distilled water was inserted in the LV cavity and used for the measurement of contractile parameters. The LV end-diastolic pressure was adjusted at 7 to 8 mm Hg. A computer using specialized software (IOX; Emka Technologies, Paris, France) allowed for analysis and recording of the LV pressure signal.

The LV functional parameters determined under isometric conditions were the left ventricular developed pressure (LVDP, mm Hg) and the positive and negative first derivative of LVDP; +dp/dt (mm Hg/sec), −dp/dt (mm Hg/sec). Measurements were obtained at the end of the 30 minutes perfusion period.

Protein Isolation, Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis, and Immunodetection

Homogenization of LV tissue and protein isolation were carried out as described previously.[17,18] The bicinchoninic acid assay method was used for determination of protein concentrations in samples.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis was performed in 7.5%, 10%, or 12% (w/v) acrylamide gels with Bio-Rad Mini Protean gel apparatus (Hercules, California). Following electrophoresis, transfer of proteins was accomplished to nitrocellulose membrane (Hybond ECL Amersham, GE Healthcare, Wauwatosa, Wisconsin). Filters were incubated with specific antibodies against sarcoplasmic Ca^{2+}-ATPase (SERCA; Affinity Bioreagents [Pierce Biotechnology, Rockford, Illinois], MA3-919, dilution 1:1000, o/n at 4°C), phospholamban (PLB; Affinity Bioreagents (Pierce Biotechnology, Rockford, Illinois), MA3-922, dilution 1:1000, o/n at 4°C), total AMPK and phospho(Thr172)-AMPK, total Akt and phospho-Akt(Ser473), total mammalian target of rapamycin (mTOR), and phospho (Ser2448)-mTOR (Cell Signaling Technology [Danvers, Massachusetts], dilution 1:1000), total and phospho(Thr202, Tyr204)-extracellular signal-regulated kinases (ERKs, Cell Signaling Technology, dilution 1:1000), total and phospho(Thr180, Tyr182)-p38 mitogen-activated protein kinase (p38 MAPK), and total and phospho(Thr183, Tyr185)-c-Jun N-terminal kinases (JNKs; Cell Signaling Technology, dilution 1:1000) overnight at 4°C. Antimouse (Amersham, GE Healthcare, Wauwatosa, Wisconsin) or antirabbit (Cell Signaling) horseradish peroxidase-conjugated secondary antibodies were used. Application of Lumiglo reagents (New England Biolabs, Whitby, Ontario) induced enhanced chemiluminescence. Analysis was performed with 5 samples per group. Fluorchem HD2 Densitometer (Alpha Innotech Corporation, California) was used for quantification of signal. Results were expressed as the ratio of phosphorylated kinase levels to total kinase levels according to common practice. Normalization of slight variations in protein loading in SERCA and PLB analysis was achieved by Ponceau staining.

Measurement of Glucose and Insulin

Insulin (ng/mL) was measured in serum using a rat/mouse insulin enzyme-linked immunosorbent assay (ELISA; Merck Millipore, Billerica, Massachusetts, EZRMI-13K) according to manufacturer's instructions. Measurements were performed with TecanGenios ELISA reader (Tecan Austria GmbH, Austria). The sensitivity of the method is 0.2 ng/mL while intra-assay variation was 2% to 7%. Glucose (in mg/dL) was measured in serum using the Amplex red glucose assay kit (Molecular Probes Europe, Leiden, Netherlands). Measurements were performed at 563 nm with Tecan Genios ELISA reader (Tecan Austria). Insulin and glucose levels were measured in serum samples from all groups: SHAM (n = 9), MI (n = 9), MI + RAN (n = 10), DM-SHAM (n = 9), DM-MI (n = 10), and DM-MI + RAN (n = 12).

Analysis of Data and Statistics

Results are presented as mean ± standard error of the mean. Kolmogorov-Smirnov test was used to estimate the normality pattern in distribution of variables. Homogeneity of variance was tested with Levene test. Multiple comparisons of variables with normal distribution were performed with 1-way analysis of variance with Bonferroni or Dunnett formula. Multiple comparisons of variables without normal distribution were made with Kruskal-Wallis nonparametric test. When statistical significance was achieved with Kruskal-Wallis test, post hoc analysis was further executed with Mann-Whitney test followed by Bonferroni correction. Analysis of qualitative parameters (incidence of pulmonary congestion) was performed with chi-square (χ^2) test and Pearson equation. Significance was set at $P < .05$.

Results

Animal Characteristics and Extent of Myocardial Injury

Animal BW was comparable in all groups (452 ± 14 g for SHAM, 434 ± 16 g for MI and 428 ± 11 g for MI + RAN, 406 ± 20 g for DM-SHAM, 422 ± 14 g for DM-MI, and 430 ± 13 g for DM-MI + RAN, $P > .05$). Furthermore, there was no difference in scar area and scar weight between the various MI groups (Table 1).

Left Ventricular Hypertrophy

In nondiabetic hearts, the ratio of left ventricular weight to BW (LVW/BW) was found to be significantly increased in the untreated infarcted rats. Ranolazine treatment resulted in significant reduction in LVW/BW, $P < .05$. In diabetic animals, there was no difference in the ratio of LVW/BW between DM-SHAM, DM-MI, and DM-MI + RAN hearts (Table 1).

Left ventricular posterior wall thickness was also found to be reduced in MI + RAN hearts at 4 weeks when compared to MI, $P < .05$, while no difference was seen between the DM-MI and DM-MI + RAN.

Effect of RAN on Serum Glucose and Insulin Levels

Serum glucose levels were normal and comparable in nondiabetic groups (108 ± 5 mg/dL in SHAM, 105 ± 5 mg/dL in MI, and 111 ± 2 mg/dL in MI + RAN, $P > .05$). Serum

Table 1. Scar Area (in mm^2) and Scar Weight (in mg), Heart Rate (in Beats/min), and Echocardiographic Measurements in Sham-Operated Rats (SHAM), Postinfarcted Hearts (MI), and Postinfarcted Hearts From Rats Treated With Ranolazine (MI + RAN) as well as in Hearts From Diabetic Sham-Operated Rats (DM-SHAM), Postinfarcted Diabetic Hearts (DM-MI), and Postinfarcted Diabetic Hearts From Rats Treated With Ranolazine (DM-MI + RAN).

	SHAM, n = 12	MI, n = 14	MI + RAN, n = 17	DM-SHAM, n = 12	DM-MI, n = 13	DM-MI + RAN, n = 15
Scar area, mm^2	–	100 ± 6.3	103.3 ± 4.7	–	101 ± 5.6	101 ± 4.3
Scar weight, mg	–	214 ± 28	189 ± 18	–	250 ± 22	234 ± 16
Heart rate, beats/min	375 ± 14	345 ± 17	381 ± 15	325 ± 22	338 ± 19	371 ± 13
EF%	78 ± 3.4	30.5 ± 1.7[a]	38.1 ± 1.8[b]	69 ± 3.1	32 ± 1.5[c]	41 ± 1.4[d]
SVPW, mm/sec	36 ± 1.6	21.5 ± 1.4[a]	26.7 ± 1.6[a]	30 ± 1.8	22 ± 1.1[c]	28 ± 1.6
LVIDd, mm	6.5 ± 0.2	9.6 ± 0.2[a]	9.2 ± 0.3[a]	6.1 ± 0.2	9.5 ± 0.4[c]	9.3 ± 0.2[c]
LVIDs, mm	3.6 ± 0.3	8.2 ± 0.25[a]	7.6 ± 0.30[a]	3.5 ± 0.2	7.8 ± 0.4[c]	7.4 ± 0.2[c]
LVPW thickness, mm	1.75 ± 0.03	2.0 ± 0.04[a]	1.8 ± 0.04[e]	1.80 ± 0.03	1.80 ± 0.06	1.89 ± 0.05
LVW/BW, mg/g	1.72 ± 0.04	2.1 ± 0.08[a]	1.8 ± 0.06[e]	1.95 ± 0.07	2.1 ± 0.08	2.09 ± 0.08

Abbreviations: LV, left ventricle; LVIDd, left ventricular internal diameter at end-diastolic phase; LVIDs, left ventricular internal diameter at end-systolic phase; EF%, ejection fraction; LVPW, left ventricular posterior wall; SVPW, Systolic velocity of posterior wall; LVW, left ventricular weight; BW, body weight; MI, myocardial infarction; RAN, ranolazine.
[a] $P < .05$ versus SHAM.
[b] $P = .07$ versus MI.
[c] $P < .05$ versus DM-SHAM.
[d] $P < .05$ versus DM-MI and DM-SHAM.
[e] $P < .05$ versus MI.

glucose levels were significantly higher in DM-SHAM (147 ± 11 mg/dL) and DM-MI (142 ± 7 mg/dL) rats when compared to nondiabetic rats, $P < .05$. Ranolazine treatment significantly reduced serum glucose levels in DM-MI + RAN rats (118 ± 4 mg/dL) compared to the levels in DM-SHAM (147 ± 11 mg/dL) and DM-MI (142 ± 7 mg/dL) rats, $P < .05$.

Serum insulin levels were comparable in nondiabetic groups (8.9 ± 0.5 ng/mL in SHAM, 8.0 ± 0.9 ng/mL in MI, and 7.5 ± 0.8 ng/mL in MI + RAN, $P > .05$). In STZ-treated rats, serum insulin levels were significantly decreased in DM-SHAM (2.7 ± 0.5 ng/mL) and DM-MI (3.1 ± 0.4 ng/mL) rats compared to those in nondiabetic rats, $P < .05$. Ranolazine treatment significantly increased serum insulin levels in DM-MI + RAN rats (5.5 ± 0.5 ng/mL) compared to those in DM-SHAM and DM-MI rats, $P < .05$.

Echocardiographic Analysis

Heart rate was comparable in all groups (Table 1). Ranolazine had no significant effect on LVIDd and LVIDs either in diabetic or in nondiabetic hearts. Ranolazine treatment had no significant effect on the SVPW radial displacement either in diabetic or in nondiabetic hearts. Left ventricular EF (LVEF%) was increased in MI + RAN as compared to MI, $P = .07$. A significant less decrease was observed in DM-MI + RAN hearts as compared to DM-MI, $P < .05$ (Table 1).

Isolated Heart Preparations: Cardiac Functional Analysis

Streptozotocin administration resulted in the development of diabetic cardiomyopathy with predominant diastolic dysfunction seen in LV function parameters. The LVDP and −dp/dt were 106 ± 1.4 mm Hg and 1931 ± 73 mm Hg/sec in DM-SHAM compared to 123 ± 3 mm Hg and 2365 ± 46 mm Hg/sec in SHAM group, respectively, $P < .05$. Left ventricular +dp/dt was not significantly different between DM-SHAM (3750 ± 82 mm Hg/sec) and SHAM (4234 ± 255 mm Hg/sec) hearts, $P > .05$ (Figure 2).

After coronary ligation, LVDP was significantly reduced in MI hearts (57 ± 6.5 mm Hg, 53% ± 5% reduction compared to SHAM) and there was a nonsignificant improvement with RAN (78 ± 7 mm Hg, 36% ± 6% reduction compared to SHAM). In the diabetic group, LVDP was significantly reduced in DM-MI (54 ± 7.3 mm Hg, 50% ± 7% reduction compared to DM-SHAM) and was significantly improved with RAN treatment (87 ± 3 mm Hg, 18% ± 3% reduction compared to DM-SHAM; Figure 2).

Left ventricular +dp/dt, a measure of systolic function, was significantly reduced in MI hearts (1940 ± 243 mm Hg/sec, 54% ± 6% reduction compared to SHAM) and was slightly less reduced in the RAN-treated group (2280 ± 200 mm Hg/sec, 46% ± 5% reduction compared to SHAM). In the diabetic group, +dp/dt was significantly reduced in DM-MI (1808 ± 320 mm Hg/sec, 52% ± 8% reduction compared to DM-SHAM) and was significantly improved in the RAN-treated group (2818 ± 144 mm Hg/sec, 25% ± 3% reduction compared to DM-SHAM; Figure 2).

Left ventricular −dp/dt, a measure of diastolic function, was significantly reduced after MI (1110 ± 144 mm Hg/sec, 53% ± 6% reduction compared to SHAM) and was slightly improved in the RAN-treated group (1541 ± 132 mm Hg, 34% ± 6% reduction compared to SHAM). In the diabetic hearts, −dp/dt was significantly reduced in DM-MI (1056 ± 143 mm Hg/sec, 47% ± 7% reduction compared to DM-SHAM) and was significantly improved in the RAN-treated group (1712 ± 74 mm Hg/sec, 12% ± 4% reduction compared to DM-SHAM; Figure 2).

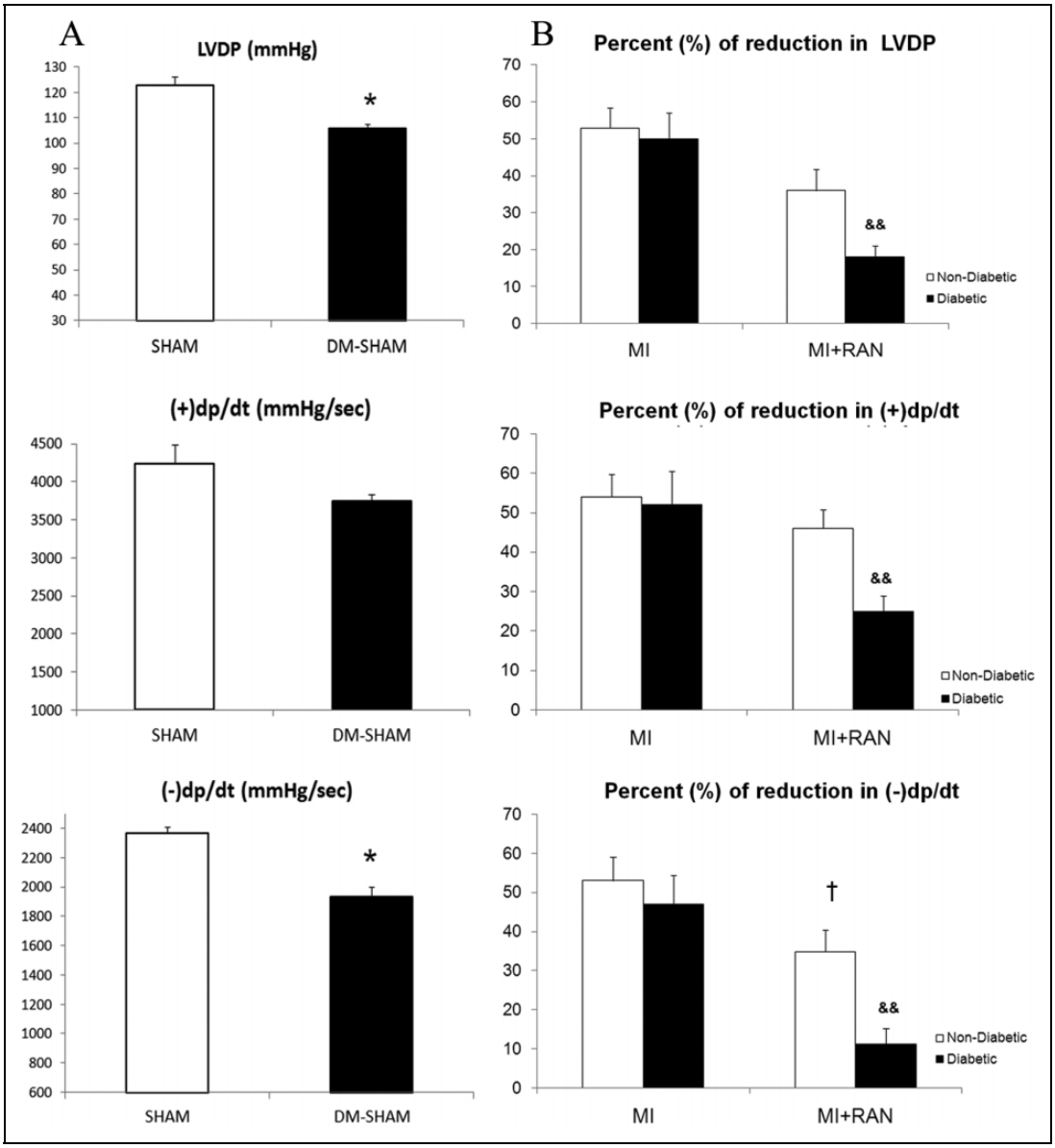

Figure 2. Hearts from a number of rats from each group were perfused according to the Langendorff technique (SHAM, n = 7; MI, n = 8; MI + RAN, n = 9; DM-SHAM, n = 7; DM-MI, n = 8; and DM-MI + RAN, n = 10). Left ventricular developed pressure (LVDP), the rate of increase of LVDP (+dp/dt), and the rate of decrease of LVDP (−dp/dt) measured under isometric conditions are shown. A, Baseline functional parameters in nondiabetic and diabetic sham-operated groups. B, Percentage of reduction in all functional parameters after MI in nondiabetic and diabetic hearts with and without RAN treatment (in comparison to the respective sham-operated groups). *$P < .05$ versus SHAM, †$P < .05$ versus MI, and &&$P < .05$ versus DM-MI and MI + RAN. DM indicates diabetes mellitus; MI, myocardial infarction; RAN, ranolazine.

Effect of RAN on Pulmonary Congestion

After coronary ligation, the ratio of LW/BW was significantly increased in MI rats (4.9 ± 0.4 compared to 3.8 ± 0.11 in SHAM, $P < .05$). No significant difference was observed between MI + RAN and MI (4.5 ± 0.3 vs 4.9 ± 0.4, $P > .05$). In addition, the incidence of pulmonary congestion was 28% (4 of 14 rats) in MI compared to 18% (3 of 17 rats) in MI + RAN group, $P > .05$ (Figure 3).

The ratio of LW/BW was significantly increased in DM-MI rats (5.2 ± 0.4 vs 4.0 ± 0.14 in DM-SHAM, $P < .05$). Ranolazine resulted in improvement in the ratio of LW/BW (4.3 ± 0.28 in DM-MI + RAN vs 5.2 ± 0.4 in DM-MI, $P = .06$). Furthermore, the incidence of pulmonary congestion was 54% (7 of 13 rats) in DM-MI group, compared to 20% (3 of 15 rats) in DM-MI + RAN group, $P < .05$ (Figure 3).

Effect of RAN on SERCA and PLB Expression

The ratio between SERCA and PLB was 1.7-fold greater in MI + RAN compared to MI hearts but this difference did not reach statistical significance (Figure 4A). In DM-MI + RAN

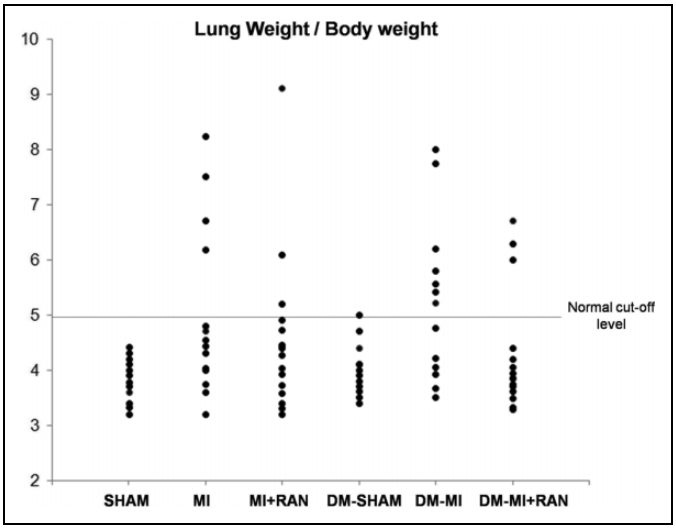

Figure 3. Scatterplots of the lung weight to body weight (LW/BW) ratio in nondiabetic sham-operated rats (SHAM, n = 12), postinfarcted (MI, n = 14), and postinfarcted rats treated with ranolazine (MI + RAN, n = 17) and in diabetic sham-operated rats (DM-SHAM, n = 12), diabetic postinfarcted rats (DM-MI, n = 13), and diabetic postinfarcted rats treated with ranolazine (DM-MI + RAN, n = 15). The ratio of LW/BW was used to assess the incidence of pulmonary congestion after myocardial infarction. Mean value of LW/BW in SHAM group ± (3 × SD) corresponding to the maximal 99% confidence interval was set as a cutoff point. DM indicates diabetes mellitus; MI, myocardial infarction; RAN, ranolazine; SD, standard deviation.

hearts, the ratio between SERCA and PLB was 2.3-fold greater compared to DM-MI groups, $P < .05$ (Figure 4B).

Effect of RAN on Stress-Induced Intracellular Kinase Signaling

The ratio of p-p38 MAPK/total p38 MAPK, the ratio of p-p54 JNK/total p54 JNK, and the ratio of p-p44/total p44 ERK and p-p42/total p42 ERK were not significantly different among SHAM, MI, and MI + RAN groups, $P > .05$. Similarly, in diabetic hearts, the ratio of p-p38 MAPK/total p38 MAPK, the ratio of p-p54 JNK/total p54 JNK, and the ratio of p-p44/total p44 ERK and p-p42/total p42 ERK were not significantly different among DM-SHAM, DM-MI, and DM-MI + RAN groups, $P > .05$.

The ratio of p-Akt/total Akt remained unchanged in MI compared to that in SHAM hearts, $P > .05$, but was increased 1.5-fold in hearts from MI + RAN compared to that in the MI group, $P < .05$ (Figure 5A). In diabetic hearts, the ratio of p-Akt/total Akt was 2-fold lower in DM-MI compared to that in DM-SHAM hearts, $P < .05$, and 1.6-fold greater in DM-MI + RAN compared to that in DM-MI hearts, $P < .05$ (Figure 5B).

The ratio of p-mTOR/mTOR was not significantly different in MI compared to SHAM hearts, $P > .05$, but it was 1.5-fold greater in MI + RAN hearts compared to that in MI, $P < .05$. In diabetic hearts, the ratio of p-mTOR/total mTOR was 1.7-fold smaller in DM-MI compared to that in DM-SHAM hearts, but this difference did not reach statistical significance. However, in DM-MI + RAN hearts, the ratio of p-mTOR/total mTOR was 2.0-fold greater compared to that in DM-MI hearts, $P < .05$.

The ratio of p-AMPK/total AMPK was not significantly different among SHAM, MI, and MI + RAN groups, $P > .05$ (Figure 6A). In diabetic hearts, the ratio of p-AMPK/total AMPK was also found to be similar in DM-MI compared to that in DM-SHAM hearts. However, in DM-MI + RAN hearts, p-AMPK/total AMPK was found to be 2.0-fold greater compared to that in both DM-SHAM and DM-MI hearts, $P < .05$ (Figure 6B).

Discussion

The present study shows that RAN, an inhibitor of late sodium current, improves the recovery of function of the postinfarcted myocardium, and this effect appears to be greater in the diabetic myocardium.

Diabetes was induced by low-dose STZ administration as described previously.[16,22] The use of higher doses of STZ is associated with heart failure and severe ketonuria (unpublished data from our laboratory). Low-dose STZ resulted in lower insulin and increased glucose levels in serum without ketonuria. Low-dose STZ treatment led to diastolic dysfunction, which is a characteristic abnormality in diabetic patients.[23] This experimental model is of clinical relevance for both type 1 and type 2 diabetes. The STZ-induced diabetes can result in insulin resistance as this occurs in patients.[24] Furthermore, insulin treatment is common in patients with type 2 diabetes due to pancreatic failure.

An experimental model of acute myocardial infarction in rats induced by permanent left coronary artery ligation with and without STZ-induced diabetes was used in this study. Acute myocardial infarction results in depressed cardiac function due to myocardial injury and structural, molecular, and functional changes, which occur in the nonischemic myocardium, known as cardiac remodeling.[20,25,26] In the present study, coronary ligation resulted in higher early mortality in diabetic than in nondiabetic animals as previously reported.[27] This is probably due to profound heart slowing and sudden death.[27] In the survived animals, myocardial injury and cardiac dysfunction after coronary ligation were comparable between the diabetic and nondiabetic animals probably due to increased tolerance of the diabetic heart to myocardial injury.[28-31] Coronary ligation resulted in cardiac hypertrophy (as assessed by echocardiographic posterior wall thickness and the ratio of left ventricular weight to BW) in nondiabetic and not in diabetic animals. This finding is consistent with previous reports.[16,22]

In this experimental setting, we explored whether RAN treatment during postinfarction period could improve recovery of function. Acute RAN treatment has been previously shown to be protective in ischemic conditions.[5] The RAN treatment started 24 hours after permanent coronary ligation to rule out the acute effect of RAN on tissue injury. The dose used resulted in average drug plasma levels of 1500 ng/mL, which are clinically relevant.[19] This treatment continued for 4 weeks. The RAN

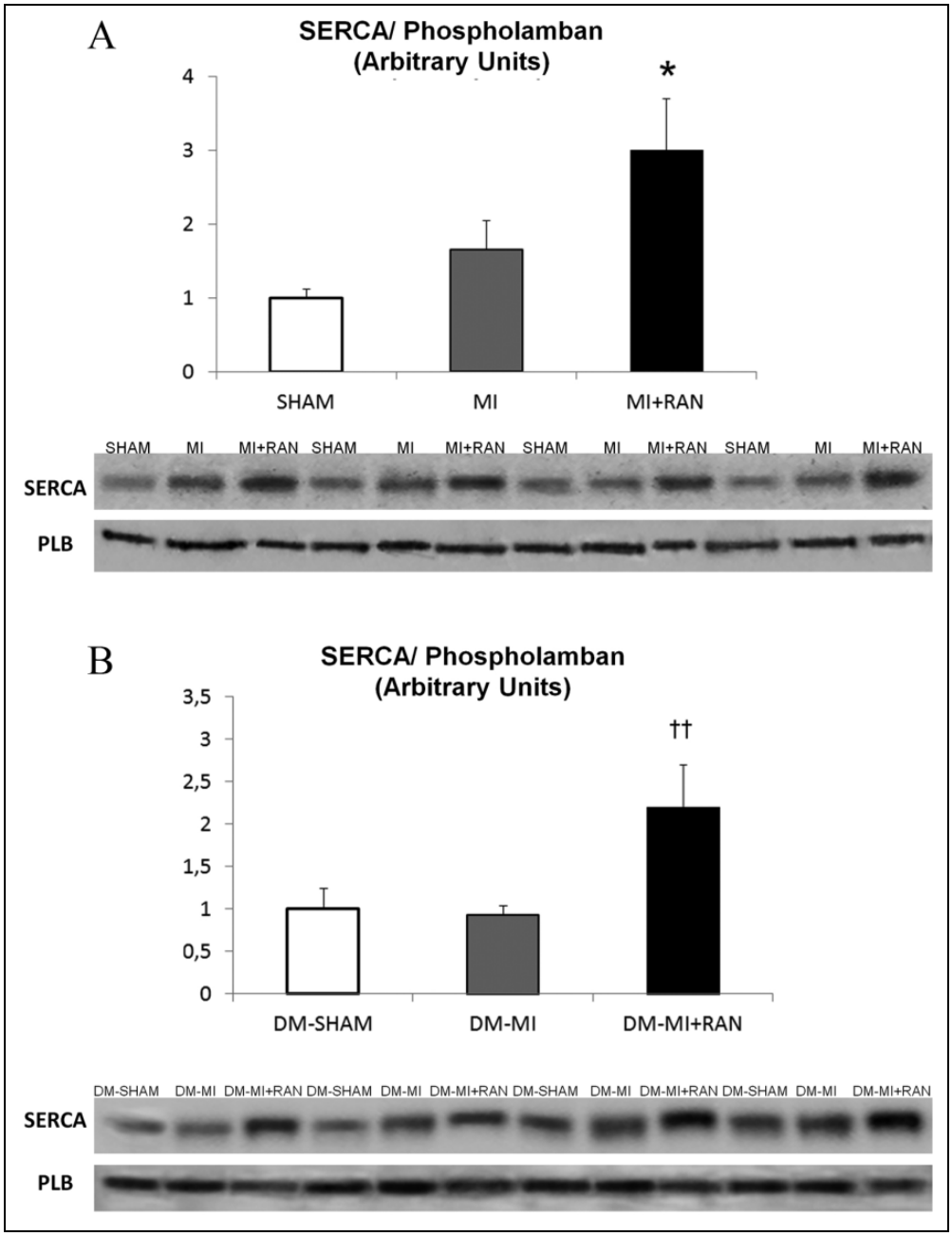

Figure 4. Summary of the densitometry ratio (mean ± standard error of the mean [SEM]) of expression of sarcoplasmic Ca^{2+}-ATPase (SERCA) and phospholamban and their representative immunoblots in (A) hearts from nondiabetic sham-operated rats (SHAM, n = 5), postinfarcted (MI, n = 5), and postinfarcted rats treated with ranolazine (MI + RAN, n = 5) and (B) hearts from diabetic sham-operated rats (DM-SHAM, n = 5), diabetic postinfarcted hearts (DM-MI, n = 5), and diabetic postinfarcted hearts of rats treated with ranolazine (DM-MI + RAN, n = 5). *P < .05 versus SHAM and $^{\dagger\dagger}P$ < .05 versus DM-MI. DM indicates diabetes mellitus; MI, myocardial infarction; RAN, ranolazine.

treatment resulted in comparable myocardial injury (as assessed by measurements of the scar area) between treated and nontreated groups. Ranolazine prevented the development of hypertrophy seen in the infarcted nondiabetic animals and had no effect on cardiac mass in the infarcted diabetic animals. Ranolazine improved the recovery of function in both nondiabetic and diabetic postinfarcted hearts and this effect was shown to be greater and achieve statistical significance only in the diabetic group. Thus, in the diabetic postinfarcted hearts, the decrease in echocardiographic LVEF% was shown to be significantly less in RAN-treated hearts. Contractile function under isometric conditions was also assessed in a number of animals according to Langendorff technique. Langendorff model has several limitations related to balloon properties, perfusion mode, oxygen dilution, and perfusate solutions. However, Langendorff model has been extensively used to study ischemia–reperfusion injury and assess cardiac function independent of loading conditions.[32] In the present study, the reduction in LVDP, +dp/dt, and −dp/dt

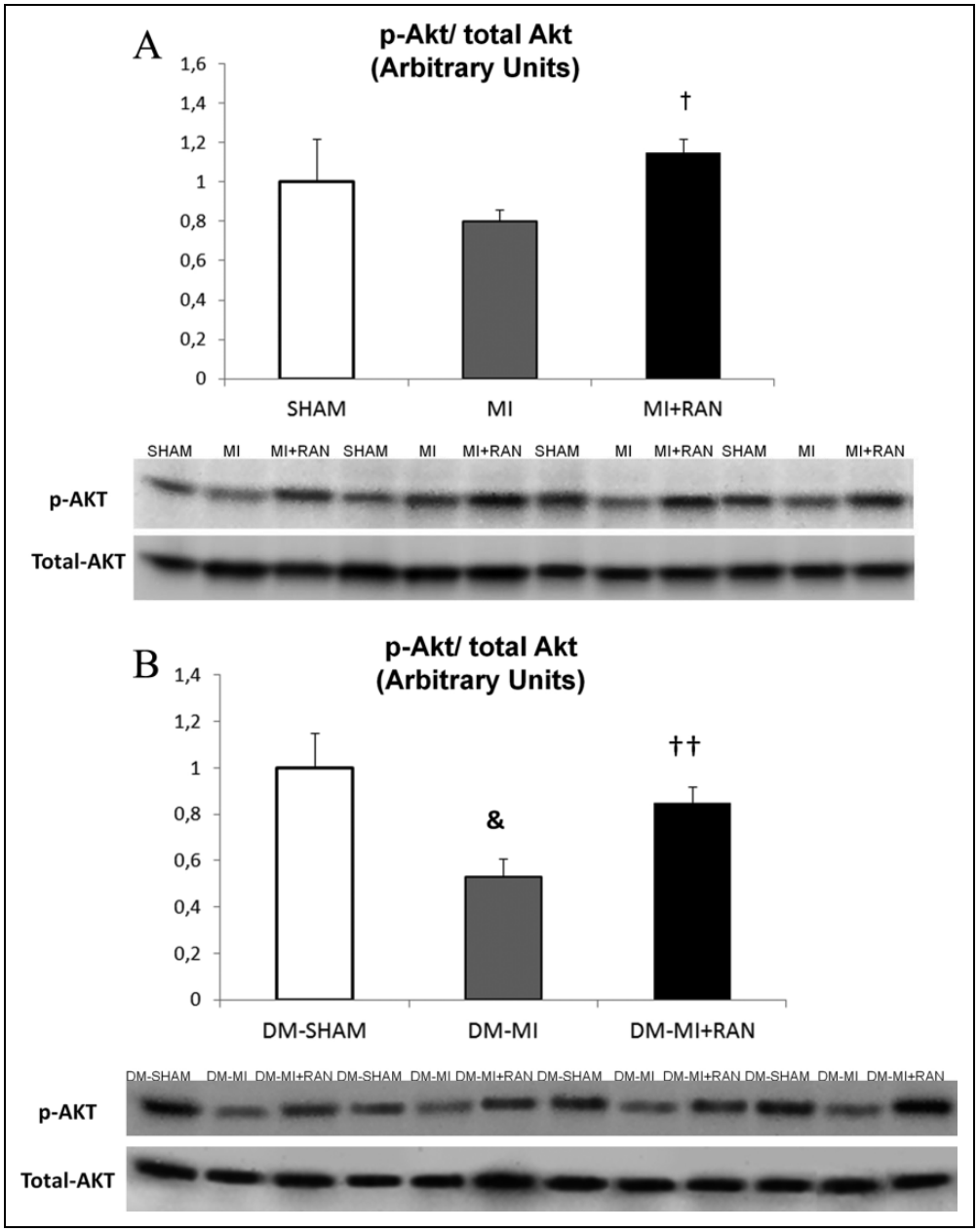

Figure 5. Summary of the densitometry ratio (mean ± standard error of the mean [SEM]) of expression of phosphorylated Akt to total Akt and their representative immunoblots in (A) hearts from nondiabetic sham-operated rats (SHAM, n = 5), postinfarcted hearts (MI, n = 5), and post-infarcted heart of rats treated with ranolazine (MI + RAN, n = 5) and (B) diabetic sham-operated rats (DM-SHAM, n = 5), diabetic postinfarcted hearts (DM-MI, n = 5), and diabetic postinfarcted hearts of rats treated with ranolazine (DM-MI + RAN, n = 5) after 4 weeks. $^{\dagger}P < .05$ versus MI, $^{\&}P < .05$ versus DM-SHAM, and $^{\dagger\dagger}P < .05$ versus DM-MI. DM indicates diabetes mellitus; MI, myocardial infarction; RAN, ranolazine.

after coronary ligation was found to be significantly less in the diabetic group treated with RAN (Figure 2). Along this line, RAN treatment improved the ratio of LW/BW (borderline significance) and significantly reduced the incidence of pulmonary congestion in the diabetic animals. Taken together, these data indicate that RAN improves the functional recovery of the post-infarcted myocardium with a greater effect in the diabetic ischemic myocardium. Consistent with these experimental data, RAN treatment is shown to result in greater antianginal effect in patients with CAD and higher HbA1c.[10]

The mechanisms underlying the greater benefit of RAN in diabetic versus nondiabetic patients are not fully understood. Nevertheless, it is likely that RAN may alter the cellular response to stress by improving calcium homeostasis. In this regard, calcium acts as an intracellular signal[33] and can alter stress-induced intracellular kinase signaling, which is critical for the cellular response to stress.[12,34,35] Calcium has been shown to alter the activation of Akt and MAPK kinases via calcium-regulated kinases and/or phosphatases in a concentration-dependent manner and thus determine cell survival/repair or death after different

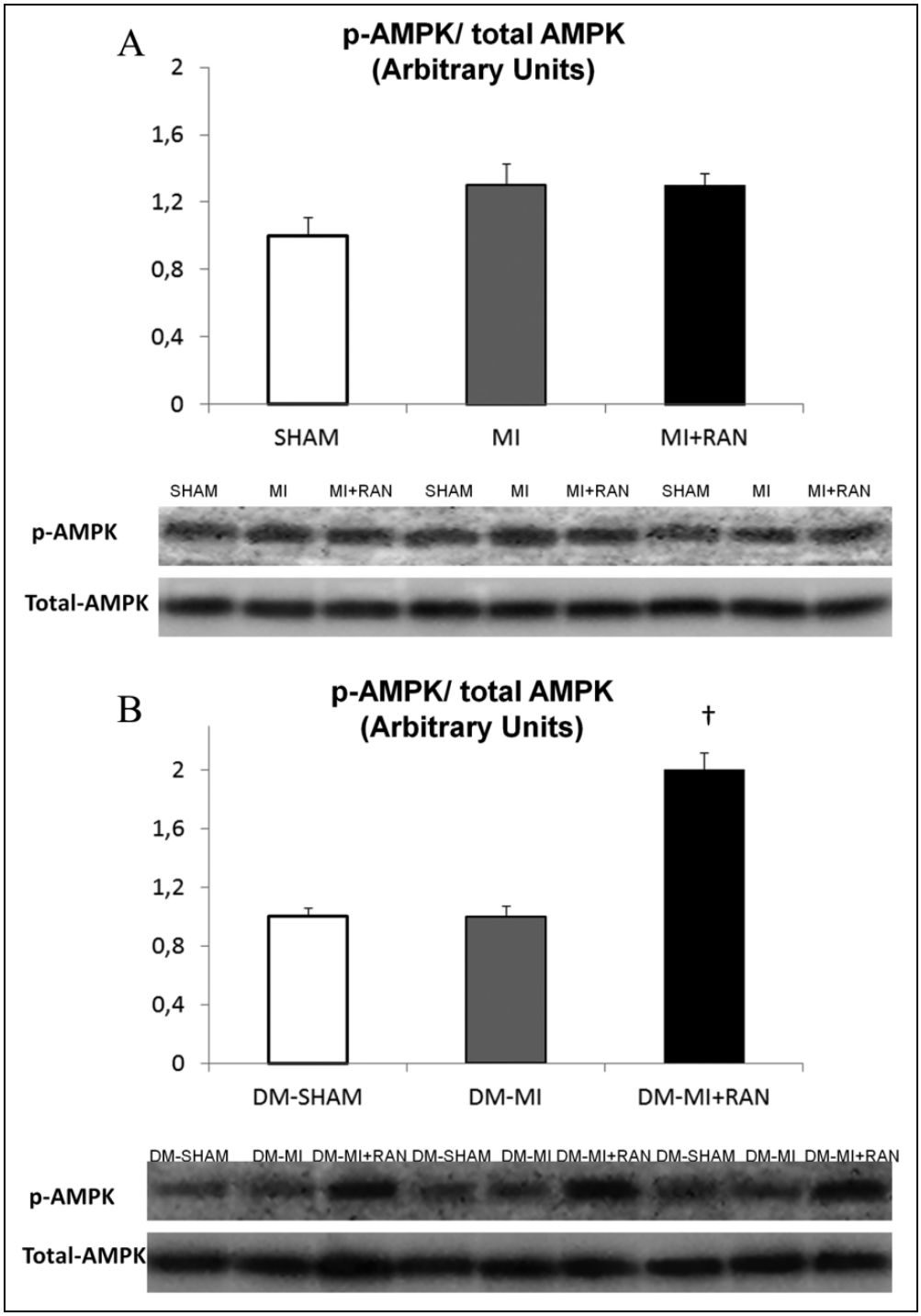

Figure 6. Summary of the densitometry ratio (mean ± standard error of the mean [SEM]) of expression of phosphorylated AMPK to total AMPK and their representative immunoblots in (A) hearts from nondiabetic sham-operated rats (SHAM, n = 5), postinfarcted (MI, n = 5), and postinfarcted hearts from rats treated with ranolazine (MI + RAN, n = 5) and (B) hearts from diabetic sham-operated rats (DM-SHAM, n = 5), diabetic postinfarcted (DM-MI, n = 5), and diabetic postinfarcted hearts from rats treated with ranolazine (DM-MI + RAN, n = 5) after 4 weeks. $^{\dagger}P < .05$ versus DM-SHAM and DM-MI. DM indicates diabetes mellitus; MI, myocardial infarction; RAN, ranolazine.

insults.[11,12,36] A link between calcium and Akt activation has been established in an H9c2 cell-based model of oxidative stress.[12] In this model, calcium overload caused suppression of Akt activation and cell death while controlled calcium concentration increased Akt activation and cell survival. Interestingly, this effect was shown to be due to calcium-dependent regulation of phosphatase gene transcription.[12] In line with this evidence, we found that phosphorylated Akt levels were not increased after acute myocardial infarction in nondiabetic animals and were suppressed in diabetic animals. The RAN treatment resulted in

significant increase in the levels of phosphorylated Akt/mTOR in both nondiabetic and diabetic hearts, probably due to its modulatory action on calcium homeostasis.[5,6] The RAN treatment had no effect on the levels of phosphorylated MAPKs (JNKs, p38 MAPK, and ERKs).

Protein kinase B is an important component of insulin signaling and regulates various downstream molecules that control function, metabolism, and cell survival.[37] Protein kinase B has a regulatory action on contractile proteins[14,15]. Interestingly, insulin increases SERCA/PLB ratio via Akt in a dose-dependent manner.[38] In the present study, RAN was shown to increase insulin levels in serum, lower glucose levels, and increase Akt/mTOR activation in the diabetic group. Furthermore, SERCA/PLB ratio was found to be significantly increased in the diabetic postinfarcted heart. The effect of RAN on glucose homeostasis has been demonstrated in patients with diabetes and CAD.[8,9,39] The mechanisms, which underlie RAN effect on insulin secretion, are not fully understood and are under investigation. The RAN is shown to have an antiapoptotic action and preserve β cells in pancreas.[39] However, other mechanisms related to its action on sodium channel may also be implicated. Inhibition of late sodium current in β-cells may increase the concentration of intracellular ATP by lowering the ATP consumption.[40] This leads to the inhibition of I_{KATP} channel and depolarization of β-cells followed by activation of calcium channels, increase intracellular calcium concentration, and consequent increase in insulin release.[41]

Taken together, our data show that RAN induces distinct changes in cell signaling in the diabetic postinfarcted heart, which probably explains the greater response of those hearts to RAN treatment. Upregulation of SERCA/PLB in the diabetic myocardium may result in further improvement in calcium homeostasis with an impact on cardiac function. Changes in SERCA/PLB ratio are closely linked to changes in calcium handling and cell contractile function.[42] Furthermore, improved calcium homeostasis may have an impact on calcium-regulated cell signaling. In fact, in the present study, calcium-regulated AMPK was found to be significantly activated in the diabetic postinfarcted heart after RAN treatment. This is of physiological relevance regarding cardiac function and metabolism. Activation of AMPK can enhance cardiomyocyte contraction and prolong relaxation by increasing Ca^{2+} sensitivity of the contractile myofilament.[43] Activation of AMPK by metformin improved left ventricular function in heart failure.[44] The AMPK also plays an important regulatory role in cardiac metabolism particularly upon stress.[45] The AMPK-regulated and Akt-dependent enhancement of glucose uptake is essential for ischemic preconditioning (IPC) beneficial effect on myocardial injury.[46] The IPC antiapoptotic effect was markedly blunted in STZ-treated diabetic rats and insulin supplementation significantly improved glucose uptake via coactivation of myocardial AMPK and Akt and limited myocardial injury.[46] Similarly, the beneficial effect of glucose–insulin–potassium treatment in patients undergoing aortic valve replacement was associated with AMPK and Akt activation in human myocardium.[47] It seems that coactivation of AMPK and Akt upon stress may enhance protection of the injured myocardium. Collectively, these data may offer, at least in part, an explanation for the greater response of the diabetic postinfarcted heart to RAN treatment (Figure 7).

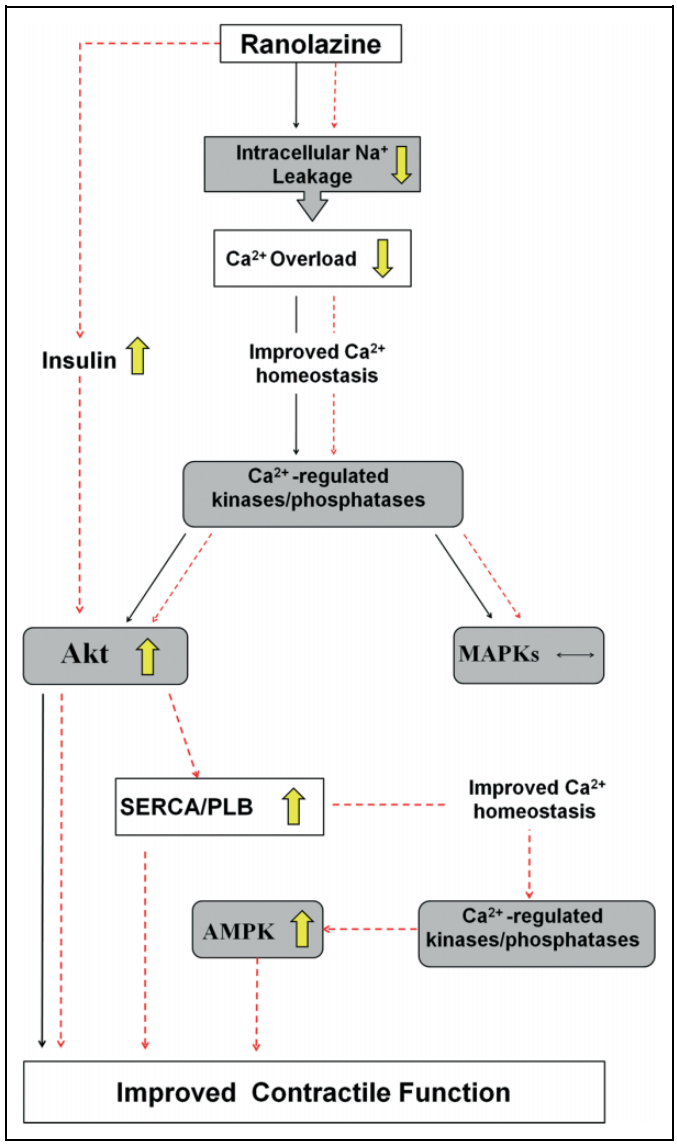

Figure 7. Schematic showing the proposed mechanism of the RAN effect on nondiabetic (black arrows) and diabetic (red arrows) postinfarcted myocardium. The RAN treatment alters stress-induced intracellular signaling with important physiological consequences on cardiac function of the stressed myocardium. RAN, ranolazine.

Clinical Relevance

Previous experimental and clinical studies have shown that RAN can protect against ischemic injury and exert antianginal effect in patients with CAD.[5,6,9,10] The present study further shows that RAN can improve the recovery of function after acute myocardial infarction. This is of clinical and therapeutic relevance. In fact, despite current available treatments, the percentage of patients without functional recovery after myocardial infarction remains relatively high and leads to future

development of heart failure.[48,49] Furthermore, our study demonstrated that RAN treatment has favorable effects on the recovery of function particularly in diabetic animals with myocardial infarction. Diabetes and heart failure are very prevalent and therapies aiming to reduce postmyocardial infarction remodeling, which progresses in heart failure, are needed for the management of this high-risk group of patients.[50] The present study offers a plausible explanation for the results of the recently published TERISA trial, indicating a better antianginal effect of RAN in patients with CAD and poorly controlled diabetes (higher HbA1c).[10] Finally, potential effects of RAN on nonischemic myocardium, particularly in the diabetic animals, were not explored. This was beyond the aim of the present study and probably merits further investigation in future studies.

In conclusion, RAN treatment after myocardial infarction improves recovery of function, and this effect appears to be greater in diabetic than nondiabetic rats. This response is associated with activation of insulin-regulated Akt/mTOR and of AMPK.

Authors' Note

This work was done at Department of Pharmacology, University of Athens, Greece, and Gilead Sciences Inc, CA, USA.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article. A.K. Dhalla and L. Belardinelli are employees of Gilead Sciences, Inc.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship and/or publication of this article: This work was supported by a grant from MENARINI INTERNATIONAL OPERATIONS LUXEMBOURG SA.

References

1. Chiha M, Njeim M, Chedrawy EG. Diabetes and coronary heart disease: a risk factor for the global epidemic. *Int J Hypertens*. 2012;2012:697240.
2. Donahoe SM, Stewart GC, McCabe CH, et al. Diabetes and mortality following acute coronary syndromes. *JAMA*. 2007;298(7):765-775.
3. Jacoby RM, Nesto RW. Acute myocardial infarction in the diabetic patient: pathophysiology, clinical course and prognosis. *J Am Coll Cardiol*. 1992;20(3):736-744.
4. Carrabba N, Valenti R, Parodi G, Santoro GM, Antoniucci D. Left ventricular remodeling and heart failure in diabetic patients treated with primary angioplasty for acute myocardial infarction. *Circulation*. 2004;110(14):1974-1979.
5. Aldakkak M, Camara AK, Heisner JS, Yang M, Stowe DF. Ranolazine reduces Ca^{2+} overload and oxidative stress and improves mitochondrial integrity to protect against ischemia reperfusion injury in isolated hearts. *Pharmacol Res*. 2011;64(4):381-392.
6. Fraser H, Belardinelli L, Wang L, Light PE, McVeigh JJ, Clanachan AS. Ranolazine decreases diastolic calcium accumulation caused by ATX-II or ischemia in rat hearts. *J Mol Cell Cardiol*. 2006;41(6):1031-1038.
7. Scirica BM, Braunwald E, Belardinelli L, et al. Relationship between nonsustained ventricular tachycardia after non-ST-elevation acute coronary syndrome and sudden cardiac death: observations from the metabolic efficiency with ranolazine for less ischemia in non-ST-elevation acute coronary syndrome-thrombolysis in myocardial infarction 36 (MERLIN-TIMI 36) randomized controlled trial. *Circulation*. 2010;122(5):455-462.
8. Chisholm JW, Goldfine AB, Dhalla AK, et al. Effect of ranolazine on A1C and glucose levels in hyperglycemic patients with non-ST elevation acute coronary syndrome. *Diabetes Care*. 2010;33(6):1163-1168.
9. Morrow DA, Scirica BM, Chaitman BR, et al. Evaluation of the glycometabolic effects of ranolazine in patients with and without diabetes mellitus in the MERLIN-TIMI 36 randomized controlled trial. *Circulation*. 2009;119(15):2032-2039.
10. Kosiborod M, Arnold SV, Spertus JA, et al. Evaluation of ranolazine in patients with type 2 diabetes mellitus and chronic stable angina. results from the TERISA randomized clinical trial. *J Am Coll Cardiol*. 2013;61(20):2038-2045.
11. Ishitani T, Kishida S, Hyodo-Miura J, et al. The TAK1-NLK mitogen-activated protein kinase cascade functions in the Wnt-5a/Ca(2+) pathway to antagonize Wnt/beta-catenin signaling. *Mol Cell Biol*. 2003;23(1):131-139.
12. Yasuoka C, Ihara Y, Ikeda S, Miyahara Y, Kondo T, Kohno S. Antiapoptotic activity of Akt is down-regulated by Ca^{2+} in myocardiac H9c2 cells. Evidence of Ca(2+)-dependent regulation of protein phosphatase 2Ac. *J Biol Chem*. 2004;279(49):51182-51192.
13. Zaha VG, Young LH. AMP-activated protein kinase regulation and biological actions in the heart. *Circ Res*. 2012;111(6):800-814.
14. Cittadini A, Monti MG, Iaccarino G, et al. Adenoviral gene transfer of Akt enhances myocardial contractility and intracellular calcium handling. *Gene Ther*. 2006;13(1):8-19.
15. Kim YK, Kim SJ, Yatani A, et al. Mechanism of enhanced cardiac function in mice with hypertrophy induced by overexpressed. *Akt J Biol Chem*. 2003;278(48):47622-47628.
16. Kalofoutis C, Mourouzis I, Galanopoulos G, et al. Thyroid hormone can favorably remodel the diabetic myocardium after acute myocardial infarction. *Mol Cell Biochem*. 2010;345(1-2):161-169.
17. Pantos C, Mourouzis I, Markakis K, et al. Thyroid hormone attenuates cardiac remodeling and improves hemodynamics early after acute myocardial infarction in rats. *Eur J Cardiothorac Surg*. 2007;32(2):333-339.
18. Pantos C, Mourouzis I, Markakis K, Tsagoulis N, Panagiotou M, Cokkinos DV. Long-term thyroid hormone administration reshapes left ventricular chamber and improves cardiac function after myocardial infarction in rats. *Basic Res Cardiol*. 2008;103(4):308-318.
19. Jerling M. Clinical pharmacokinetics of ranolazine. *Clin Pharmacokinet*. 2006;45(5):469-491.
20. Pantos C, Mourouzis I, Galanopoulos G, et al. Thyroid hormone receptor alpha1 downregulation in postischemic heart failure

progression: the potential role of tissue hypothyroidism. *Horm Metab Res*. 2010;42(10):718-724.
21. Pantos C, Mourouzis I, Saranteas T, et al. Thyroid hormone improves postischaemic recovery of function while limiting apoptosis: a new therapeutic approach to support hemodynamics in the setting of ischaemia–reperfusion? *Basic Res Cardiol*. 2009; 104(1):69-77.
22. Mourouzis I, Giagourta I, Galanopoulos G, et al. Thyroid hormone improves the mechanical performance of the post-infarcted diabetic myocardium: a response associated with up-regulation of Akt/mTOR and AMPK activation. *Metabolism*. 2013;62(10):1387-1393.
23. Boudina S, Abel ED. Diabetic cardiomyopathy revisited. *Circulation*. 2007;115(25):3213-3223.
24. Hall KE, McDonald MW, Grise KN, Campos OA, Noble EG, Melling CW. The role of resistance and aerobic exercise training on insulin sensitivity measures in STZ-induced type 1 diabetic rodents. *Metabolism*. 2013;62(10):1485-1494.
25. Burchfield JS, Xie M, Hill JA. Pathological ventricular remodeling: mechanisms: part 1 of 2. *Circulation*. 2013;128(4): 388-400.
26. Mourouzis I, Kostakou E, Galanopoulos G, Mantzouratou P, Pantos C. Inhibition of thyroid hormone receptor alpha1 impairs post-ischemic cardiac performance after myocardial infarction in mice. *Mol Cell Biochem*. 2013;379(1-2):97-105.
27. Luo M, Guan X, Luczak ED, et al. Diabetes increases mortality after myocardial infarction by oxidizing CaMKII. *J Clin Invest*. 2013;123(3):2333-2374.
28. Ma G, Al-Shabrawey M, Johnson JA, et al. Protection against myocardial ischemia/reperfusion injury by short-term diabetes: enhancement of VEGF formation, capillary density, and activation of cell survival signaling. *Naunyn Schmiedebergs Arch Pharmacol*. 2006;373(6):415-427.
29. Pantos C, Mourouzis I, Cokkinos DV. Protection of the abnormal heart. *Heart Fail Rev*. 2007;12(3-4):319-330.
30. Tsang A, Hausenloy DJ, Mocanu MM, Carr RD, Yellon DM. Preconditioning the diabetic heart: the importance of Akt phosphorylation. *Diabetes*. 2005;54(8):2360-2364.
31. Xu G, Takashi E, Kudo M, Ishiwata T, Naito Z. Contradictory effects of short- and long-term hyperglycemias on ischemic injury of myocardium via intracellular signaling pathway. *Exp Mol Pathol*. 2004;76(1):57-65.
32. Bell RM, Mocanu MM, Yellon DM. Retrograde heart perfusion: the Langendorff technique of isolated heart perfusion. *J Mol Cell Cardiol*. 2011;50(6):940-950.
33. Molkentin JD. Dichotomy of Ca^{2+} in the heart: contraction versus intracellular signaling. *J Clin Invest*. 2006;116(3):623-626.
34. Latronico MV, Costinean S, Lavitrano ML, Peschle C, Condorelli G. Regulation of cell size and contractile function by AKT in cardiomyocytes. *Ann N Y Acad Sci*. 2004;1015:250-260.
35. Wang Y. Mitogen-activated protein kinases in heart development and diseases. *Circulation*. 2007;116(12):1413-1423.
36. Takeda K, Matsuzawa A, Nishitoh H, et al. Involvement of ASK1 in Ca2+-induced p38 MAP kinase activation. *EMBO Rep*. 2004; 5(2):161-166.
37. Hers I, Vincent EE, Tavare JM. Akt signalling in health and disease. *Cell Signal*. 2011;23(10):1515-1527.
38. Fredersdorf S, Thumann C, Zimmermann WH, et al. Increased myocardial SERCA expression in early type 2 diabetes mellitus is insulin dependent: in vivo and in vitro data. *Cardiovasc Diabetol*. 2012;11:57.
39. Ning Y, Zhen W, Fu Z, et al. Ranolazine increases beta-cell survival and improves glucose homeostasis in low-dose streptozotocin-induced diabetes in mice. *J Pharmacol Exp Ther*. 2011;337(1): 50-58.
40. Ding WG, Kitasato H. Suppressing Na^{+} influx induces an increase in intracellular ATP concentration in mouse pancreatic beta-cells. *Jpn J Physiol*. 1997;47(3):299-306.
41. Iezzi M, Kouri G, Fukuda M, Wollheim CB. Synaptotagmin V and IX isoforms control Ca^{2+}-dependent insulin exocytosis. *J Cell Sci*. 2004;117(pt 15):3119-3127.
42. Kranias EG, Hajjar RJ. Modulation of cardiac contractility by the phospholamban/SERCA2a regulatome. *Circ Res*. 2012;110(12): 1646-1660.
43. Oliveira SM, Zhang YH, Solis RS, et al. AMP-activated protein kinase phosphorylates cardiac troponin I and alters contractility of murine ventricular myocytes. *Circ Res*. 2012; 110(9):1192-1201.
44. Gundewar S, Calvert JW, Jha S, et al. Activation of AMP-activated protein kinase by metformin improves left ventricular function and survival in heart failure. *Circ Res*. 2009;104(3):403-411.
45. Arad M, Seidman CE, Seidman JG. AMP-activated protein kinase in the heart: role during health and disease. *Circ Res*. 2007;100(4): 474-488.
46. Ji L, Zhang X, Liu W, et al. AMPK-regulated and Akt-dependent enhancement of glucose uptake is essential in ischemic preconditioning-alleviated reperfusion injury. *PLoS One*. 2013; 8(7):e69910.
47. Howell NJ, Ashrafian H, Drury NE, et al. Glucose-insulin-potassium reduces the incidence of low cardiac output episodes after aortic valve replacement for aortic stenosis in patients with left ventricular hypertrophy: results from the Hypertrophy, Insulin, Glucose, and Electrolytes (HINGE) trial. *Circulation*. 2011; 123(2):170-177.
48. Lymvaios I, Mourouzis I, Cokkinos DV, et al. Thyroid hormone and recovery of cardiac function in patients with acute myocardial infarction: a strong association? *Eur J Endocrinol*. 2011;165(1):107-114.
49. Springeling T, Kirschbaum SW, Rossi A, et al. Late cardiac remodeling after primary percutaneous coronary intervention. *Circ J*. 2012;77(1):81-88.
50. Yin M, Westenbrink B, Meissner M, van Gilst WH, de Boer RA. Variable effects of anti-diabetic drugs in animal models of myocardial ischemia and remodeling: a translational perspective for the cardiologist. *Int J Cardiol*. 2013;169(6):385-393.