The Insulin Sensitizer Pioglitazone Improves the Deterioration of Ischemic Preconditioning in Type 2 Diabetes Mellitus Rats

Hideki SASAKI,¹ MD, Kazuhiko OGAWA,¹ MD, Mitsuyuki SHIMIZU,¹ MD, Chikara MORI,¹ MD, Hisashi TAKATSUKA,¹ MD, Fumiko OKAZAKI,¹ MD, Makoto KAWAI,¹ MD, Ikuo TANIGUCHI,¹ MD, *and* Seibu MOCHIZUKI,¹ MD

SUMMARY

We investigated the effects of ischemic preconditioning (IP) on reperfusion arrhythmias in type 2 diabetic rats as well as the effects of the insulin sensitizer pioglitazone. Thirty-week-old OLETF rats with or without pioglitazone (10 mg/kgBW, orally) were used as a model for type 2 diabetes. LETO rats served as controls. The incidences and durations of reperfusion ventricular tachyarrhythmias (RVT) were evaluated using a working heart method. After 5 minutes of initial perfusion, the rats were divided into the following groups: 1) control rats without IP (CIP(-)), 2) control rats with IP (CIP(+)), 3) diabetic rats without IP (DIP(-)), 4) diabetic rats with IP (DIP(+)), 5) pioglitazone-treated diabetic rats without IP (TDIP(-)), and 6) pioglitazone-treated diabetic rats with IP (TDIP(+)). Three 2-minute cycles of global diastolic ischemia and 5 minutes of reperfusion before long ischemia were performed as IP. The incidence and duration of RVT in CIP(+) were significantly lower than in CIP(-). There was no significant difference in the duration of RVT between DIP(+) and DIP(-). However, the duration of RVT in TDIP(+) was significantly shorter than TDIP(-). These results suggested that the effects of IP on reperfusion arrhythmias are deteriorated in type 2 diabetic rats. The insulin sensitizer pioglitazone can improve the deterioration of IP against reperfusion arrhythmias in type 2 diabetic rats. (Int Heart J 2007; 48: 623-635)

Key words: Otsuka Long Evans Tokushima Fatty (OLETF) rat, Ischemia, Reperfusion arrhythmias, Insulin sensitizer, Pioglitazone, Ischemic preconditioning, Type 2 diabetes mellitus

DIABETES mellitus is an important coronary risk factor, and it is well known that diabetic patients with ischemic heart disease have a very poor prognosis.¹⁻³⁾ However, it has been reported that the hearts of diabetic animal models possess tolerance to ischemic injury.⁴⁻¹⁴⁾

From the ¹ Division of Cardiology, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan.

Address for correspondence: Hideki Sasaki, MD, Division of Cardiology, Department of Internal Medicine, The Jikei University School of Medicine, 3-25-8 Nishi-Shinbashi, Minato-ku, Tokyo 105-8461, Japan.

Received for publication May 21, 2007.

Revised and accepted July 5, 2007.

Ischemic preconditioning (IP) is a well known phenomenon preceding a brief episode of myocardial ischemia and it increases ischemic tolerance and prevents myocardial dysfunction caused by subsequent sustained ischemic insult.¹⁵⁾ The absence of the beneficial effects of IP in diabetic animals has been reported.¹⁶⁻¹⁸⁾ However, in diabetic animal models the effects of IP on reperfusion arrhythmias have not yet been clearly demonstrated.¹⁹⁾

An autoimmune spontaneously diabetic rat, a non-autoimmune spontaneously diabetic rat, and a drug (STZ)-induced diabetic animal model have been used as experimental diabetic animal models. The Otsuka Long Evans Tokushima Fatty (OLETF) rat is a non-autoimmune spontaneously diabetic animal model, and has been reported to develop hypertriglyceridemia, obesity, and insulin resistance as well as human type 2 diabetes.²⁰⁾ However, there are few reports concerning myocardial tolerance to ischemia and IP using OLETF rats.^{21,22)}

Pioglitazone is the only thiazolidinedione currently available in Japan. Recently, PROactive,²³⁾ a randomized controlled trial, showed that pioglitazone reduced cardiovascular mortality in patients with type 2 diabetes who have a high-risk of macrovascular events. It is believed that an improvement in insulin resistance is very important for type 2 diabetic patients, however, the mechanism of the reduction of cardiovascular events in PROactive remains unclear.

In this study, we evaluated the effects of IP, especially on reperfusion arrhythmias, using isolated OLETF rat hearts and a working heart method. We also evaluated whether administration of the insulin sensitizer pioglitazone had any influence on the effects of IP on isolated OLETF rat hearts.

METHODS

This study was conducted in accordance with the guidelines of the Institution Guidance Committee of the Jikei University School of Medicine for the Use and Care of Animals.

Experimental animals: Thirty-week-old OLETF rats²⁰⁾ were used as a spontaneously type 2 diabetes animal model while Long Evans Tokushima Otsuka (LETO) rats served as controls. Pioglitazone (10 mg/kg body weight/day, orally) was administered to OLETF rats for 1 month to induce pioglitazone-treated diabetes.

Isolated rat heart perfusion: The rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg). The heart was promptly removed and perfused by the Langendorff method, and then converted to a working heart method (preload 10 cmH₂O, afterload 80 cmH₂O) as previously described.²⁴⁾ Modified Krebs-Henseleit bicarbonate buffer (K-H buffer, mM: NaCl 118, KCl 4.7, CaCl₂ 2.0, MgSO₄ 1.2, KH₂PO₄ 1.2, EDTA 0.5, NaHCO₃ 25, glucose 11, pH

Figure 1. Perfusion protocol. IP indicates ischemic preconditioning.

7.4, 37°C, 95%O₂+5%CO₂ gas mixture) was used as the perfusion buffer. A one-way ball valve in the cannula inserted into the aorta was used to block coronary perfusion during diastole in order to produce ischemia. Electrical pacing (300 bpm, 3V) was performed during ischemia.

Perfusion protocol (Figure 1): After 5 minutes of initial perfusion, the rats were divided into the following groups: 1) control rats without IP (CIP(-); *n* = 5), 2) control rats with IP (CIP(+); *n* = 6), 3) diabetic rats without IP (DIP(-); *n* = 6), 4) diabetic rats with IP (DIP(+); *n* = 6), 5) pioglitazone-treated diabetic rats without IP (TDIP(-); *n* = 6), and 6) pioglitazone-treated diabetic rats with IP (TDIP(+); *n* = 6). As IP, 3 cycles of global ischemia for 2 minutes (2-minute ischemia) and reperfusion for 5 minutes (5-minute reperfusion) were performed before long ischemia (10-minute ischemia) was performed.

Experimental parameters and measurement of lactate and H⁺ in the coronary effluent: Electrocardiograms were recorded with carbon leads directly attached to the perfused heart surface and monitored during these protocols. Reperfusion ventricular tachyarrhythmias (RVT) were defined as ventricular tachycardia, ventricular flutter, and ventricular fibrillation. Arrhythmias were analyzed according to the Lambeth Convention Criteria.²⁵⁾

Coronary flow was monitored by inserting a cannula into the pulmonary artery. P_{O₂}, P_{CO₂}, [HCO₃⁻], pH, and lactate were measured using a Blood Gas Analyzer (Corning 175, USA). [H⁺] was calculated with values of P_{CO₂} and [HCO₃⁻] according to the following formula.

$$[H^+] \text{ (nmol/L)} = 24 \times P_{CO_2} \text{ (mmHg)} / [HCO_3^-] \text{ (nmol/L)}$$

Figure 2. Body weights and HbA_{1c} levels. **A:** Body weight (g). Values are means $\pm$ SEM. **B:** HbA_{1c} level (%). Values are means $\pm$ SEM.

Statistical analysis: The χ^2 -test was used to compare the incidences of RVT. All data are expressed as the mean $\pm$ SEM. Student's *t*-test was used to compare two groups, and one-factor ANOVA was used to compare more than 3 groups. *P* values < 0.05 were considered to indicate statistical significance.

RESULTS

Body weight (Figure 2A): Body weight in diabetic rats (682 ± 39 g) and pioglitazone-treated diabetic rats (715 ± 61 g) was significantly higher than in control rats (507 ± 27 g) ($P < 0.01$). There was no significant difference in body weight between diabetic rats and pioglitazone-treated diabetic rats.

HbA_{1c} (Figure 2B): HbA_{1c} in diabetic rats ($3.82 \pm 0.47\%$) was significantly higher than in control rats ($2.84 \pm 0.14\%$) ($P < 0.01$), and HbA_{1c} in pioglitazone-treated diabetic rats ($2.98 \pm 0.33\%$) was significantly lower than in diabetic rats ($P < 0.05$).

Incidence rates of RVT: In groups without IP, there was no significant difference in the incidence rate of RVT among CIP(-) (5/5;100%), DIP(-) (5/6;83.3%), and TDIP(-) (6/6;100%).

In control rats, the incidence rate of RVT in CIP(+)(2/6;33.3%) was significantly lower than CIP(-) ($P < 0.05$).

In diabetic rats, there was no significant difference in the incidence rate of RVT between DIP(+)(4/6;66.7%) and DIP(-).

In pioglitazone-treated diabetic rats, there was no significant difference in

the incidence rate of RVT between TDIP(+)(5/6;83.3%) and TDIP(-).

Duration of RVT (Figure 3): In groups without IP, there was no significant difference in the duration of RVT among CIP(-) (17.7 ± 2.0 minutes), (DIP(-) (16.5 ± 3.6 minutes), and TDIP(-) (17.9 ± 2.1 minutes).

In control rats, the duration of RVT in CIP(+)(6.7 ± 4.6 minutes) was significantly lower than CIP(-)($P < 0.05$)(Figure 3A).

Figure 3. Duration of reperfusion ventricular tachyarrhythmia **A:** CIP(-) and CIP(+). Values are means $\pm$ SEM. CIP(-) indicates control rats without IP; and CIP(+), control rats with IP. **B:** DIP(-) and DIP(+). Values are means $\pm$ SEM. DIP(-) indicates diabetic rats without IP; and DIP(+), diabetic rats with IP. **C:** TDIP(-) and TDIP(+). Values are means $\pm$ SEM. TDIP(-) indicates treated diabetic rats without IP; and TDIP(+), treated diabetic rats with IP.

Figure 4. Coronary flow in control rats. Values are means $\pm$ SEM. CIP(-) indicates control rats without IP (circle); and CIP(+), control rats with IP (square). * $P < 0.05$

In diabetic rats, there was no significant difference in the duration of RVT between DIP(+) (13.3 ± 4.6 minutes) and DIP(-) (Figure 3B).

In pioglitazone-treated diabetic rats, the duration of RVT in TDIP(+)(4.9 ± 1.7 minutes) was significantly lower than TDIP(-)($P < 0.001$) (Figure 3C).

Changes in coronary flow (Figures 4-6): In controls rats, coronary flow decreased from 100 mL/min. to approximately 20 mL/min. after ischemia, and recovered to baseline flow soon after reperfusion. The changes in coronary flow were similar in the 3 cycles of 2-minute ischemia and the 5-minute reperfusion. Coronary flow

Figure 5. Coronary flow in diabetic rats. Values are means $\pm$ SEM. DIP(-) indicates diabetic rats without IP (circle); and DIP(+), diabetic rats with IP (square). * $P < 0.05$

Figure 6. Coronary flow in treated diabetic rats. Values are means $\pm$ SEM. TDIP(-) indicates treated diabetic rats without IP (circle); and TDIP(+), treated diabetic rats with IP (square). * $P < 0.05$

was 0 mL/min. during 10-minute ischemia, increased to 120-140 mL/min. soon after reperfusion, and recovered to 90-100 mL/min at 12 minutes after reperfusion. The coronary flow for 4 minutes after reperfusion in CIP(+) was significantly higher than in CIP(-) ($P < 0.05$).

The changes in coronary flow in DIP(+) and TDIP(+) were similar to CIP(+). There was no significant difference in the change in coronary flow between DIP(+) and DIP(-). However, the coronary flow at 12 minutes after reperfusion in TDIP(+) was significantly higher than in TDIP(-) ($P < 0.05$).

Changes in $[H^+]$ in coronary effluent (Figures 7-9): In groups without IP, $[H^+]$ in coronary effluent in CIP(-) increased from 40 nmol/L to approximately 110 nmol/L after 10-minute ischemia, and decreased after reperfusion. There was no significant difference in the change in $[H^+]$ in coronary effluent among CIP(-), DIP(-), and TDIP(-).

In control rats $[H^+]$ in coronary effluent in CIP(+) was approximately 40 nmol/L, increased significantly during 2-minute ischemia ($P < 0.05$) of IP, and recovered soon after reperfusion. The more frequent the brief ischemia and reperfusion were, the less $[H^+]$ in coronary effluent increased. $[H^+]$ in effluent increased to approximately 80 nmol/L during 10-minute ischemia and decreased soon after reperfusion. $[H^+]$ in coronary effluent during 10-minute ischemia in CIP(+) was significantly lower than in CIP(-) ($P < 0.05$).

In diabetic rats, there was no significant increment of $[H^+]$ in coronary effluent during 2-minute ischemia. $[H^+]$ in coronary effluent during 10-minute ischemia increased to approximately 60 nmol/L, and $[H^+]$ in coronary effluent in DIP(+) during 10-minute ischemia and after reperfusion was significantly lower than in DIP(-) ($P < 0.05$).

Figure 7. $[H^+]$ and lactate in coronary effluent in control rats. Values are means $\pm$ SEM. CIP(-) indicates control rats without IP (circle); and CIP(+), control rats with IP (square). * $P < 0.05$

In pioglitazone-treated diabetic rats, $[H^+]$ in coronary effluent was significantly increased both during 2-minute ischemia ($P < 0.05$) and during 10-minute ischemia. However, $[H^+]$ in coronary effluent soon after reperfusion in TDIP(+) was lower than in TDIP(-) ($P < 0.05$).

Changes in lactate levels in coronary effluent (Figures 7-9): Lactate levels in coronary effluent in control rats, diabetic rats, and pioglitazone-treated diabetic rats increased significantly during ischemia, and decreased soon after reperfusion. There was no significant difference in the change in lactate level among the 3 groups. In all 3 groups, IP significantly inhibited the increment of lactate level during ischemia ($P < 0.05$). There was no significant difference in the inhibition

Figure 8. $[H^+]$ and lactate in coronary effluent in diabetic rats. Values are means $\pm$ SEM. DIP(-) indicates diabetic rats without IP (circle); and DIP(+), diabetic rats with IP (square). * $P < 0.05$

Figure 9. $[H^+]$ and lactate in coronary effluent in treated diabetic rats. Values are means $\pm$ SEM. TDIP(-) indicates diabetic rats without IP (circle); and TDIP(+), diabetic rats with IP (square). * $P < 0.05$

of the increment of lactate level by IP among the 3 groups.

DISCUSSION

Usefulness of OLETF rats as a spontaneously type 2 diabetic animal model: The OLETF rats used in this study are a type 2 diabetic animal model produced by selective breeding of rats from the Long-Evans strain, and have serum glucose levels of 300 mg/dL or greater at 60 minutes in an oral glucose tolerance test. It has been reported that OLETF rats develop hyperinsulinemia, insulin resistance, and diabetes mellitus.^{20,26)}

In this study, body weight and HbA_{1C} levels in 30-week-old OLETF rats were significantly higher than age-matched LETO rats. It is believed that 30-week-old OLETF rats are a good model of type 2 diabetes mellitus.

IP effects on RVT in diabetes mellitus: Arrhythmias that develop due to reperfusion after ischemia are also an ischemia/reperfusion injury. The mechanisms of reperfusion arrhythmias may be involved in heterogeneous recovery of conduction and refractory period by incomplete reperfusion, reentry, abnormal automaticities, triggered activities²⁷⁾ by Ca²⁺ overload,²⁸⁾ and free radicals.²⁹⁻³¹⁾ Furthermore, it has been reported that IP markedly inhibits ischemia reperfusion arrhythmias and reoxygenation arrhythmias.³²⁻³⁴⁾ The mechanisms by which IP inhibits reperfusion arrhythmias may involve the reduction of Ca²⁺ overload due to the inhibition of production of [H⁺] and lactate³⁵⁻³⁷⁾ and the opening of sarcolemmal and/or mitochondrial K_{ATP} channels.³⁸⁻⁴⁴⁾ However, the details of the mechanisms have not been clarified. In control rats, the incidence and the duration of RVT in CIP(+) were significantly lower than in CIP(-) ($P < 0.05$). These results suggested that IP in this protocol can inhibit reperfusion arrhythmias.

It has been reported that the hearts of diabetic animals possess tolerance to ischemic injury.⁴⁻¹⁴⁾ However, there was no significant difference in the incidence and duration of RVT between CIP(-) and DIP(-) in this study, suggesting that the diabetic heart used in this study does not have tolerance against reperfusion arrhythmias. Moreover, in diabetic rats there was no significant difference in the incidence and duration of RVT between DIP(+) and DIP(-). These results suggested that the effect of IP on reperfusion arrhythmias was also decreased in diabetes mellitus. The data in this study were similar to the findings of clinical reports in type 2 diabetic patients by Ishihara³⁸⁾ and Jimenez-Navarro,⁴⁵⁾ and the experimental reports by Kristiansen,⁴⁶⁾ but were different from the reports in type 1 diabetes by Tosaki⁴⁷⁾ and Ravingerova.⁴⁸⁾ The difference in the type of diabetes may be the reason for these differences.⁴⁹⁻⁵¹⁾ Therefore, the effect of IP may be decreased in type 2 diabetes mellitus with hyperinsulinemia, although it has not yet been clarified in type 1 diabetes mellitus without hyperinsulinemia.

Effects of pioglitazone: HbA_{1C} levels in diabetic rats treated with the insulin sensitizer pioglitazone were significantly lower than in diabetic rats. There was no significant difference in HbA_{1C} between control rats and pioglitazone-treated diabetic rats. These results suggested that OLETF rats had developed insulin resistance, and that pioglitazone improved insulin resistance and controlled blood glucose levels.^{20,26)}

There were no significant differences in the incidence and duration of RVT among CIP(-), DIP(-), and TDIP(-), suggesting that diabetes mellitus and/or pioglitazone did not directly affect the incidence and duration of RVT.

In diabetic rats there was no significant difference in the duration of RVT between DIP(+) and DIP(-). However, in pioglitazone-treated diabetic rats the duration of RVT in TDIP(+) was significantly lower than in TDIP(-). These results suggested that pioglitazone treatment might bring about recovery of IP effects which were decreased in type 2 diabetes mellitus. Moreover, a significant increment of [H⁺] in coronary effluent during 2-minute ischemia was not observed in diabetic rats, but it was seen in pioglitazone-treated diabetic rats, suggesting that the increment of [H⁺] during brief ischemia might play an important role in the inhibitory effects by IP against reperfusion arrhythmias, and that pioglitazone might bring about the recovery of IP effects in type 2 diabetes.

The details of the mechanisms of some of the actions of thiazolidinediones such as pioglitazone remain unclear.^{52,53)} It has been reported that thiazolidinediones not only improve insulin resistance, but also improve antiatherosclerotic actions in vessels, such as the induction of adiponectin and anti-inflammatory effects, as well as improve endothelial dysfunction.⁵⁴⁻⁶⁴⁾ Moreover, it has also been reported that thiazolidinediones have myocardial protective effects against ischemia and/or reperfusion injury.^{65,66)} However, there has never been a report about the effect of a thiazolidinedione on IP in type 2 diabetes mellitus. This study showed that the insulin sensitizer pioglitazone improved deterioration of the effects of IP against reperfusion arrhythmias in type 2 diabetes mellitus. Therefore, the treatment of insulin resistance using pioglitazone might not only control blood glucose levels, but also protect the myocardium in type 2 diabetes mellitus.

Conclusions: We conclude that the effects of IP are decreased in type 2 diabetes mellitus and that pioglitazone improves glucose metabolism and decreased IP effects in type 2 diabetes mellitus.

ACKNOWLEDGEMENTS

The authors are grateful to Takeda Pharmaceutical Company Limited (Osaka, Japan) for providing the pioglitazone used in this study.

REFERENCES

1. Jaffe AS, Spadaro JJ, Schechtman K, Roberts R, Geltman EM, Sobel BE. Increased congestive heart failure after myocardial infarction of modest extent in patients with diabetes mellitus. *Am Heart J* 1984; 108: 31-7.
2. Mak KH, Moliterno DJ, Granger CB, *et al.* Influence of diabetes mellitus on clinical outcome in the thrombolytic era of acute myocardial infarction. GUSTO-I Investigators. Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries. *J Am Coll Cardiol* 1997; 30: 171-9.
3. Haffner SM, Lehto S, Ronnema T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998; 339: 229-34.
4. Tani M, Neely JR. Hearts from diabetic rats are more resistant to in vitro ischemia: possible role of altered Ca^{2+} metabolism. *Circ Res* 1988; 62: 931-40.
5. Khandoudi N, Bernard M, Cozzone P, Feuvray D. Mechanisms of intracellular pH regulation during post-ischemic reperfusion of diabetic rat hearts. *Diabetes* 1995; 44: 196-202.
6. Gamble J, Lopaschuk GD. Glycolysis and glucose oxidation during reperfusion of ischemic hearts from diabetic rats. *Biochem Biophys Acta* 1994; 1225: 191-9.
7. Lopaschuk GD, Saddik M, Barr R, Huang L, Barker CC, Muzyka RA. Effects of high levels of fatty acids on functional recovery of ischemic hearts from diabetic rats. *Am J Physiol* 1992; 263: E1046-53.
8. Bouchard JF, Lamontagne D. Protection afforded by preconditioning to the diabetic heart against ischemic injury. *Cardiovasc Res* 1998; 37: 82-90.
9. Tatsumi T, Matoba S, Kobara M, *et al.* Energy metabolism after ischemic preconditioning in streptozotocin-induced diabetic rat hearts. *J Am Coll Cardiol* 1998; 31: 707-15.
10. Feuvray D. The regulation of intracellular pH in the diabetic myocardium. *Cardiovasc Res* 1997; 34: 48-54. (Review)
11. Kusuoka H, Matsuda S, Ishikawa M, *et al.* Difference in the mechanisms for compensating ischemic acidosis in diabetic rat hearts. *J Mol Cell Cardiol* 1998; 30: 1643-9.
12. Ramasamy R, Schaefer S. Inhibition of $\text{Na}^+\text{-H}^+$ exchanger protects diabetic and non-diabetic hearts from ischemic injury: insight into altered susceptibility of diabetic hearts to ischemic injury. *J Mol Cell Cardiol* 1999; 31: 785-97.
13. Liu Y, Thornton JD, Cohen MV, Downey JM, Schaffer SW. Streptozotocin-induced non-insulin-dependent diabetes protects the heart from infarction. *Circulation* 1993; 88: 1273-8.
14. Mozaffari MS, Schaffer SW. Effect of hypertension and hypertension-glucose intolerance on myocardial ischemic injury. *Hypertension* 2003; 42: 1042-9.
15. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986; 74: 1124-36.
16. Katakam PV, Jordan JE, Snipes JA, Tulbert CD, Miller AW, Busija DW. Myocardial preconditioning against ischemia-reperfusion injury is abolished in Zucker obese rats with insulin resistance. *Am J Physiol Regul Integr Comp Physiol* 2007; 292: R920-6.
17. Hassouna A, Loubani M, Matata BM, Fowler A, Standen NB, Galinanes M. Mitochondrial dysfunction as the cause of the failure to precondition the diabetic human myocardium. *Cardiovasc Res* 2006; 69: 450-8.
18. Tsang A, Hausenloy DJ, Mocanu MM, Carr RD, Yellon DM. Preconditioning the diabetic heart: the importance of Akt phosphorylation. *Diabetes* 2005; 54: 2360-4.
19. Ravingerova T, Stetka R, Pancza D, Ulicna O, Ziegelhoffer A, Styk J. Susceptibility to ischemia-induced arrhythmias and the effect of preconditioning in the diabetic rat heart. *Physiol Res* 2000; 49: 607-16.
20. Kawano K, Hirashima T, Mori S, Saitoh Y, Kurosumi M, Natori T. Spontaneous long-term hyperglycemic rat with diabetic complications. Otsuka Long-Evans Tokushima Fatty (OLETF) strain. *Diabetes* 1992; 41: 1422-8.
21. Maddaford TG, Russell JC, Pierce GN. Postischemic cardiac performance in the insulin-resistant JCR:LA-cp rat. *Am J Physiol* 1997; 273: H1187-92.
22. Yue TL, Bao W, Gu JL, *et al.* Rosiglitazone treatment in Zucker diabetic Fatty rats is associated with ameliorated cardiac insulin resistance and protection from ischemia/reperfusion-induced myocardial injury. *Diabetes* 2005; 54: 554-62.
23. Dormandy J, Charbonnel B, Eckland DJ, *et al.* Secondary prevention of macrovascular events in patients with

- type 2 diabetes in the PROactive Study (PROactive pioglitAzone Clinical Trial In macroVascular Events): a randomized controlled trial. *Lancet* 2005; 366: 1279-89.
24. Neely JR, Rovetto MJ, Whitmer JT, Morgan HE. Effects of ischemia on function and metabolism of the isolated working rat heart. *Am J Physiol* 1973; 225: 651-8.
 25. Sakamoto J, Miura T, Tsuchida A, Fukuma T, Hasegawa T, Shimamoto K. Reperfusion arrhythmias in the murine heart: their characteristics and alteration after ischemic preconditioning. *Basic Res Cardiol* 1999; 94: 489-95.
 26. Taniguchi Y, Ooie T, Takahashi N, *et al.* Pioglitazone but not glibenclamide improves cardiac expression of heat shock protein 72 and tolerance against ischemia/reperfusion injury in the heredity insulin-resistant rat. *Diabetes* 2006; 55: 2371-8.
 27. Spray DC, Bennett MV. Physiology and pharmacology of gap junctions. *Ann Rev Physiol* 1985; 47: 281-303. (Review)
 28. Dennis SC, Coetzee WA, Cragoe EJ Jr, Opie LH. Effects of proton buffering and amiloride derivatives on reperfusion arrhythmias in isolated rat hearts. Possible evidence for an arrhythmogenic role of $\text{Na}^+\text{-H}^+$ exchange. *Circ Res* 1990; 66: 1156-9.
 29. Manning AS, Coltart DJ, Hearse DJ. Ischemia and reperfusion-induced arrhythmias in the rat. Effects of xanthine oxidase inhibition with allopurinol. *Circ Res* 1984; 55: 545-8.
 30. Bernier M, Hearse DJ, Manning AS. Reperfusion-induced arrhythmias and oxygen-derived free radicals. Studies with "anti-free radical" interventions and a free radical-generating system in the isolated perfused rat heart. *Circ Res* 1986; 58: 331-40.
 31. Grech ED, Dodd NJ, Bellamy CM, Perry RA, Morrison WL, Ramsdale DR. Free-radical generation during angioplasty reperfusion for acute myocardial infarction. *Lancet* 1993; 341: 990-1.
 32. Heyndrickx GR, Vatner SF, Wijins W, *et al.* Stunning, hibernation, and preconditioning. *Clinical Pathophysiology of Myocardial Ischemia*. Philadelphia, USA: Lippincott-Raven; 1997.
 33. Connaughton M, Hearse DJ. Three questions about preconditioning. *Basic Res Cardiol* 1996; 91: 12-5.
 34. Kasamaki Y, Guo AC, McDonald TF. Protection by hypoxic preconditioning against hypoxia-reoxygenation injury in guinea-pig papillary muscles. *Cardiovasc Res* 1997; 34: 313-22.
 35. Taniguchi M, Onodera T, Kinoshita T, *et al.* Possible role of the preconditioning for the prevention of myocardial H^+ production. *Cardioangiography* 1993; 34: 590-6 (in Jpn).
 36. Taniguchi M, Onodera T, Kinoshita T, *et al.* Possible role of H^+ production in preconditioning for the prevention of reperfusion-induced arrhythmias. *J Clin Exp Med* 1992; 162: 953-4 (in Jpn with Eng abstr).
 37. Osada M, Sato T, Komori S, Tamura K. Protective effect of preconditioning on reperfusion induced ventricular arrhythmias of isolated rat hearts. *Cardiovasc Res* 1991; 25: 441-4.
 38. Ishihara M, Inoue I, Kawagoe T, *et al.* Diabetes mellitus prevents ischemic preconditioning in patients with a first acute anterior wall myocardial infarction. *J Am Coll Cardiol* 2001; 38: 1007-11.
 39. Noma A. ATP-regulated K^+ channels in cardiac muscle. *Nature* 1983; 305: 147-8.
 40. Miura T, Liu Y, Kia H, Ogawa T, Shimamoto K. Roles of mitochondrial ATP-sensitive K^+ channels and PKC in anti-infarct tolerance afforded by adenosine A1 receptor activation. *J Am Coll Cardiol* 2000; 35: 238-45.
 41. del Valle HF, Lascano EC, Negroni JA, Crottogini AJ. Absence of ischemic preconditioning protection in diabetic sheep hearts: role of sarcolemmal KATP channel dysfunction. *Mol Cell Biochem* 2003; 249: 21-30.
 42. Smith JM, Wahler GM. ATP-sensitive potassium channels are altered in ventricular myocytes from diabetic rats. *Mol Cell Biochem* 1996; 158: 43-51.
 43. Nakayama K, Fan Z, Marumo F, Hiraoka M. Interrelation between pinacidil and intracellular ATP concentrations on activation of the ATP-sensitive K^+ current in guinea pig ventricular myocytes. *Circ Res* 1990; 67: 1124-33.
 44. Allison TB, Brutting SP, Crass MF 3rd, Eliot RS, Shipp JC. Reduced high-energy phosphate levels in rat hearts. I. Effects of alloxan diabetes. *Am J Physiol* 1976; 230: 1744-50.
 45. Jimenez-Navarro M, Gomez-Doblas JJ, Hernandez Garcia JM, *et al.* Does angina pectoris the week before protect against first acute myocardial infarction in patients with diabetes mellitus? *Am J Cardiol* 2002; 90: 160-2.
 46. Kristiansen SB, Lofgren B, Stottrup NB, *et al.* Ischaemic preconditioning does not protect the heart in obese and lean animal models of type 2 diabetes. *Diabetologia* 2004; 47: 1716-21.
 47. Tosaki A, Engelman DT, Engelman RM, Das DK. The evolution of diabetic response to ischemia/reperfusion and preconditioning in isolated working rat hearts. *Cardiovasc Res* 1996; 31: 526-36.

48. Ravingerova T, Stetka R, Volkovova K, *et al.* Acute diabetes modulates response to ischemia in isolated rat heart. *Mol Cell Biochem* 2000; 210: 143-51.
49. Ebel D, Mullenheim J, Frassdorf J, *et al.* Effect of acute hyperglycemia and diabetes mellitus with and without short-term insulin treatment on myocardial ischaemic late preconditioning in the rabbit heart in vivo. *Pflugers Arch* 2003; 446: 175-82.
50. Marfella R, Di Filippo C, Esposito K, *et al.* Absence of inducible nitric oxide synthase reduces myocardial damage during ischemia reperfusion in streptozotocin-induced hyperglycemic mice. *Diabetes* 2004; 53: 454-62.
51. Kersten JR, Toller WG, Gross ER, Pagel PS, Warltier DC. Diabetes abolishes ischemic preconditioning: role of glucose, insulin, and osmolality. *Am J Physiol Heart Circ Physiol* 2000; 278: H1218-24.
52. Tonelli J, Li W, Kishore P, *et al.* Mechanisms of early insulin-sensitizing effects of thiazolidinediones in type 2 diabetes. *Diabetes* 2004; 53: 1621-9.
53. Tanaka T, Yamamoto J, Iwasaki S, *et al.* Activation of peroxisome proliferator-activated receptor delta induces fatty acid beta-oxidation in skeletal muscle and attenuates metabolic syndrome. *Proc Natl Acad Sci USA* 2003; 100: 15924-9.
54. Yki-Jarvinen H. Thiazolidinediones. *N Engl J Med* 2004; 351: 1106-18. (Review)
55. Kersten S, Desvergne B, Wahli W. Roles of PPARs in health and disease. *Nature* 2000; 405: 421-4. (Review)
56. Yamauchi T, Kamon J, Waki H, *et al.* The fat-derived hormone adiponectin reverses insulin resistance associated with both lipodystrophy and obesity. *Nat Med* 2001; 7: 941-6.
57. Kubota N, Terauchi Y, Kubota T, *et al.* Pioglitazone ameliorates insulin resistance and diabetes by both adiponectin-dependent and -independent pathways. *J Biol Chem* 2006; 281: 8748-55.
58. Iwaki M, Matsuda M, Maeda N, *et al.* Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes* 2003; 52: 1655-63.
59. Hirose H, Kawai T, Yamamoto Y, *et al.* Effects of pioglitazone on metabolic parameters, body fat distribution, and serum adiponectin levels in Japanese male patients with type 2 diabetes. *Metabolism* 2002; 51: 314-7.
60. Hanefeld M, Marx N, Pfützner A, *et al.* Anti-inflammatory effects of pioglitazone and/or simvastatin in high cardiovascular risk patients with elevated high sensitivity C-reactive protein: the PIOSTAT Study. *J Am Coll Cardiol* 2007; 49: 290-7.
61. Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature* 1998; 391: 82-6.
62. Hetzel J, Balletshofer B, Rittig K, *et al.* Rapid effects of rosiglitazone treatment on endothelial function and inflammatory biomarkers. *Arterioscler Thromb Vasc Biol* 2005; 25: 1804-9.
63. Wang N, Verna L, Chen NG, *et al.* Constitutive activation of peroxisome proliferator-activated receptor-gamma suppresses pro-inflammatory adhesion molecules in human vascular endothelial cells. *J Biol Chem* 2002; 277: 34176-81.
64. Sourij H, Zweiker R, Wascher TC. Effects of pioglitazone on endothelial function, insulin sensitivity, and glucose control in subjects with coronary artery disease and new-onset type 2 diabetes. *Diabetes Care* 2006; 29: 1039-45.
65. Sidell RJ, Cole MA, Draper NJ, Desrois M, Buckingham RE, Clarke K. Thiazolidinedione treatment normalizes insulin resistance and ischemic injury in the Zucker Fatty rat heart. *Diabetes* 2002; 51: 1110-7.
66. Shibata R, Sato K, Pimentel DR, *et al.* Adiponectin protects against myocardial ischemia-reperfusion injury through AMPK- and COX-2-dependent mechanisms. *Nat Med* 2005; 11: 1096-103.