Statin reverses reduction of adiponectin receptor expression in infarcted heart and in TNF-α-treated cardiomyocytes in association with improved glucose uptake

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Statin reverses reduction of adiponectin receptor expression in infarcted heart and in TNF-α-treated cardiomyocytes in association with improved glucose uptake. Am J Physiol Heart Circ Physiol 293: H3490–H3497, 2007. First published September 28, 2007; doi:10.1152/ajpheart.00310.2007.—Statin treatment improves insulin resistance in skeletal muscle. Thus this study assessed whether statin may affect the myocardial expression levels of AdipoR1 and AdipoR2, receptors of adiponectin that enhance insulin sensitivity, and whether statin may improve insulin resistance in cardiomyocytes. Myocardial infarction (MI) was created by the ligation of the left coronary artery in male mice. Expression levels of mRNA and protein levels of AdipoR1 but not of AdipoR2 were significantly decreased in the remote area as well as in the healed infarcted area in the left ventricles 4 wk after MI. Oral administration of pravastatin (50 mg·kg⁻¹·day⁻¹ for 4 wk after MI) reversed the decrease in myocardial expression levels of AdipoR1 independently of changes in serum lipid profiles and insulin levels. With the use of cultured cardiomyocytes, incubation with tumor necrosis factor (TNF)-α, a mediator of postinfarction myocardial dysfunction, inhibited AdipoR1 mRNA and protein expression levels. Coincubation of the cells with pravastatin reversed the inhibitory effects of TNF-α on AdipoR1 expression. In parallel, pravastatin reversed the TNF-α-induced decrease in globular adiponectin-induced 2-deoxy-D-[³H]glucose uptake in insulin-treated cultured cells. Moreover, this effect of pravastatin was inhibited by the suppression of AdipoR1 expression by small-interfering RNA specific for AdipoR1. Incubation with H₂O₂ reduced AdipoR1 expression in cultured cardiomyocytes that were attenuated by N-acetylcysteine or pravastatin. Pravastatin suppressed TNF-α-induced intracellular oxidants in cultured cardiomyocytes. In conclusion, pravastatin reversed the reduction of AdipoR1 expression in postinfarction mouse myocardium and in TNF-α-treated cardiomyocytes partly through an antioxidative mechanism in association with improved glucose uptake.

OVER THE PAST SEVERAL years, many pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) have been identified (10, 25). Statins improve the prognosis of heart diseases via an improvement of endothelial function and suppression of inflammatory responses in addition to lipid-lowering effects (1, 18). To date, there is compelling evidence that an impaired energy metabolism in cardiomyocytes strongly influences ventricular remodeling (13, 20). It is also known that abnormalities in glucose and lipid metabolism in cardiac muscle are associated with heart failure (5, 15). It is thus possible that drugs that improve myocardial metabolic abnormalities might be useful in preventing the progression of heart failure. Recently, it was shown that atorvastatin exerts a favorable effect on insulin sensitivity of skeletal muscle in diabetic animals (26). Therefore, it is expected that statins may improve insulin sensitivity in the myocardium of the failing heart.

Adiponectin, the most abundant protein secreted from adipose tissue, has antiatherogenic, anti-inflammatory, and antidiabetic effects (3, 4, 9, 12, 22, 24). Furthermore, circulating levels of adiponectin are lower in coronary artery disease (CAD) and predict future cardiovascular events (17). Adiponectin plays a fundamental role in glucose and lipid metabolism in adipose tissue and has insulin-sensitizing effects on the liver and skeletal muscle (4, 9, 12). Two novel adiponectin receptor types (AdipoR1 and AdipoR2) were recently identified (27). In skeletal muscle, adiponectin increases glucose uptake and fatty acid oxidation through stimulation of these receptors (9, 27). We have also demonstrated that myocardium expresses AdipoR1 and AdipoR2 and that AdipoR1 and AdipoR2 might play a possible role in the pathogenesis of endothelin-1-induced hypertrophy of cardiomyocytes after myocardial infarction (MI) (6). Thus, this study assessed the hypothesis that statin treatment may increase the expression of AdipoR1 and/or AdipoR2 in postinfarction myocardium, leading to an improvement of myocardial insulin resistance.

METHODS

Materials. Pravastatin was a gift from Sankyo (Tokyo, Japan). Rat recombinant globular adiponectin (glb-adiponectin) was purchased from Adipogen (Sungnam, Korea). Anti-AdipoR1 polyclonal antibody was purchased from Affinity BioReagents (Colden, CO), and anti-AdipoR2 polyclonal antibody was purchased from Alpha Diagnostic International (San Antonio, TX). Anti-β-tubulin polyclonal antibody was from Santa Cruz Biotechnology (Santa Cruz, CA). Cell culture reagents were from Sigma (Tokyo, Japan) and Invitrogen (Carlsbad, CA). Fetal bovine serum (FBS) and 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) were from Invitrogen. 2-Deoxy-

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d-[3H]glucose was from Moravek Biochemicals (Brea, CA). [1-14C]palmitic acid was from Perkin Elmer (Boston, MA). Rat recombinant tumor necrosis factor (TNF-α) was from BioVision Research Products (Mountain View, CA). Insulin and other chemicals were purchased from Sigma.

Animal models of MI. The experimental protocol was approved by the University of Yamanashi Animal Care and Use Committee, and all procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996). MI in 8- to 12-wk-old male mice was created by ligation of the left coronary artery under anesthesia with pentobarbital sodium (50 mg/kg ip) and ventilated with a respirator (6). The chest was then closed with 7-0 polypropylene sutures. On day 1 after MI, mice were randomized to treatment with pravastatin (50 mg·kg⁻¹·day⁻¹) or vehicle by gavage for 4 wk. Only mice with extensive MI (>50%) were analyzed for the measurement of mRNA and protein expression levels. Sham-operated mice served as controls. Finally, the mice were euthanized and harvested 4 wk after the surgery. Parts of the tissue samples from the left ventricle were quickly frozen and stored at −80°C until use for the measurement of mRNA and protein expression levels. Blood was collected by intracardiac puncture just before euthanasia under anesthesia. Serum levels of total cholesterol, triglyceride, and glucose were determined by enzymatic assays according to the protocols of the manufacturers (Wako and Sigma, Osaka, Japan). Fasting serum insulin levels were determined by an enzyme immunoassay (Merckodia, Sweden).

Preparation and culture of cardiomyocytes. Primary cultures of rat neonatal cardiomyocytes were prepared by trypsin/EDTA digestion from the ventricles of 1- to 3-day-old Sprague-Dawley rats, as described previously (6). Briefly, after trypsinization, the cells were collected by centrifugation and diluted to 5 × 10⁶ cells/ml in DMEM containing 10% FBS. The cells were preplated and cultured for 30 min to delete the nonmyocardial cells. Nonattached cells were suspended in DMEM containing 10% FBS and plated for 72 h on plastic petri dishes of the indicated sizes. After cells were washed, they were replaced with DMEM containing 0.5% FBS for 24 h before each experiment. For measurement of mRNA and protein expression levels, the cultured cells were treated for 12 or 24 h with TNF-α (10 ng/ml), norepinephrine (100 nM), H2O2 (10 mM), or the vehicle in the presence or absence of pravastatin (10 μM) in DMEM medium containing 0.5% FBS. After cells were washed twice with cold PBS, they were incubated for 30 min with or without glb-adiponectin (0.25 μg/ml) in glucose-free DMEM medium containing 0.1% fatty acid free bovine serum albumin. After subsequent incubation with insulin (10 nM) for an additional 30 min, glucose uptake was initiated by the addition of 2-deoxy-d-[3H]glucose to a final assay concentration of 3 μCi/ml for 10 min at 37°C. Reactions were terminated by rapidly washing the cells twice with cold PBS. Cardiomyocytes were solubilized in 1 N NaOH and neutralized with 1 N HCl, and the radioactivity in aliquots from cell extracts was counted by liquid scintillation (MicroScint20, Packard Instrument, Meriden, CT). Protein assay of the cell extracts was performed with 10 μl of the lysate by use of a BCA protein assay kit (Pierce Chemical, Rockford, IL). Deoxyglucose uptake was expressed as counts per minute per milligram of protein and normalized to vehicle-treated cells.

Measurement of fatty acid oxidation in cultured cardiomyocytes. Experiments for fatty acid oxidation were performed with modifications to the methods described by Yamauchi et al. (28). Cardiomyocytes were allowed to attach to 60-mm center-well organ culture dishes. The attached cells were incubated for 24 h with TNF-α (10 ng/ml) or the vehicle in the presence or absence of pravastatin (10 μM) in DMEM medium containing 0.5% FBS. Subsequently, the cells were preincubated for 6 h with or without glb-adiponectin (0.25 μg/ml). After cells were washed twice with cold PBS, they were incubated with DMEM containing 0.05 mM palmitic acid, 3% fatty acid free bovine serum albumin, and 5 mM glucose with or without glb-adiponectin (0.25 μg/ml) for 30 min at 37°C. [1-14C]Palmitic acid (0.5 μCi) was then added, and the incubation was continued for an additional 30 min to reach a steady-state situation. Thereafter, 500 ml of 0.1 N NaOH were then injected into the center wells of the dishes, and the dishes were subsequently sealed airtight. The oxidation was terminated by injecting 400 μl of 1 M H2SO4 into the incubation buffer at 30 min after the addition of NaOH. The dishes were stored at 4°C overnight. The trapping medium (NaOH) was then assayed for 14CO2 by liquid scintillation counting (Opti-Fluor, Perkin Elmer; and LSC-5100, Aloka, Tokyo, Japan). The oxidation rate was calculated by subtracting trapped 14CO2 at zero time from trapped 14CO2 after 30 min of incubation and was expressed as counts per minute per milligram of protein and normalized to vehicle-treated cells.

RNA interference and transfection. Small-interfering RNAs (siRNAs) were designed and synthesized by Invitrogen, as described in a previous report from Kugiyama’s laboratory (6). The sequences of the
sense siRNAs are listed in Table 1. The cultured cardiomyocytes were transfected with 100 nM of siRNAs by using Lipofectamine 2000 (6). After cardiomyocytes were washed, the medium was replaced with DMEM medium containing 0.5% FBS for 12 h. The cells were then assayed for glucose uptake.

Assessment of intracellular oxidants production in cultured cardiomyocytes. Intracellular reactive oxygen species (ROS) production was measured using DCF-DA (5). The cultured cardiomyocytes in 12 wells (3 × 10^5 cells/well) were treated for 6 h with TNF-α (10 ng/ml) or the vehicle in the presence or absence of pravastatin (1–10 μM), mevalonate (100 μM), NAC (1 mM), or glb-adiponec-tin (0.25 μg/ml) in DMEM containing 0.5% FBS. After being washed with PBS, the cells were subsequently loaded with DCF-DA (10 μmol/l) in PBS at 37°C for 30 min and then washed twice with PBS to remove the excess probe. Dichlorofluorescein (DCF) fluorescence intensity was immediately measured in a plate reader (Gemini EM, Molecular Devices, Sunnyvale, CA) with an excitation/emission wavelength of 495/525 nm. DCF values were calculated after subtracting background fluorescence levels (measured under identical conditions but without DCF) and expressed as values per milligram of protein. Fluorescence intensity was normalized to the vehicle-treated cells. In parallel, fluorescence images of the cultured cells were obtained from three or more randomly chosen fields using an inverted epifluorescence microscope (IX70; Olympus, Tokyo, Japan) with a 470 – 490 nm excitation and 515–550 nm emission filter (IX-FLA; Olympus). The

Fig. 1. mRNA and protein expression of adiponectin receptors 1 and 2 (AdipoR1 and AdipoR2, respectively) in the left ventricle 4 wk after myocardial infarction (MI). A: real-time quantitative PCR analysis of AdipoR1 (left) and AdipoR2 (right) mRNA expression in the remote (top) and the infarcted (bottom) areas of the left ventricle 4 wk after MI in mice. Oral pravastatin (Prava; 50 mg·kg⁻¹·day⁻¹) or vehicle was administered during 4 wk after MI. Sham-operated mice served as a control group. The levels of mRNA expression were normalized to GAPDH mRNA expression and expressed relative to those of the sham-operated ventricle. Values are expressed as means ± SE (n = 9 in each experiment). Insets show representative agarose gel electrophoresis of the amplified PCR products from 0.1 μg of total RNA (AdipoR1 and GAPDH at 23 cycles and AdipoR2 at 30 cycles). β: immunoblot analysis using antibodies against AdipoR1 (left) and AdipoR2 (right) protein expression in the remote area (top) and the infarcted area (bottom) and in the sham-operated ventricle. The intensity of the β-tubulin band was used as a loading control between samples. The protein levels are expressed relative to those of the sham-operated ventricle. Values are expressed as means ± SE (n = 9–15). *P < 0.05. NS, not significant.
statin and adiponectin receptors in cardiomyocytes

Table 2. Effects of pravastatin on serum levels of total cholesterol, triglyceride, glucose, and insulin in mice

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<th>Sham</th>
<th>MI + Vehicle</th>
<th>MI + Pravastatin</th>
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<tr>
<td>Total cholesterol, mg/dl</td>
<td>98.7±3.1</td>
<td>94.3±2.8</td>
<td>95.9±4.9</td>
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<tr>
<td>Triglyceride, mg/dl</td>
<td>68.5±6.5</td>
<td>96.4±6.5</td>
<td>92.6±8.8</td>
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<tr>
<td>Glucose, mg/dl</td>
<td>111.5±7.9</td>
<td>137.0±10.6</td>
<td>111.4±13.9</td>
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<tr>
<td>Insulin, ng/ml</td>
<td>1.47±0.43</td>
<td>1.05±0.23</td>
<td>0.93±0.19</td>
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Values are expressed as means ± SE; n = 14. Fasting serum levels 4 wk after myocardial infarction (MI) in mice. Oral pravastatin (50 mg·kg⁻¹·day⁻¹) or vehicle was administered daily during 4 wk after MI.

Comparisons among three or more groups were performed by one-way ANOVA with the Tukey’s test procedure used for post hoc comparisons of group means. P < 0.05 was considered statistically significant.

RESULTS

Effects of pravastatin treatment on expressions of AdipoR1 and AdipoR2 in hearts after MI. When compared with the normal left ventricle, expression levels of AdipoR1 mRNA and protein were decreased in the remote area as well as in the infarcted area 4 wk after MI in mice (Fig. 1). AdipoR2 expression levels had a tendency to decrease in both areas, but they did not reach statistical significance (Fig. 1). Oral administration of pravastatin attenuated the decrease in expression levels of AdipoR1 mRNA and protein in the remote area 4 wk after MI (Fig. 1). Expression levels of AdipoR2 mRNA and protein were not significantly changed by pravastatin treatment.

Fig. 2. Expression of AdipoR1 in cultured cardiomyocytes. A: real-time quantitative PCR analysis of AdipoR1 mRNA expression in neonatal-cultured cardiomyocytes after incubation for 12 h with TNF-α (10 ng/ml), nor-epinephrine (Norepi; 100 nM), or vehicle in the presence or absence of Prava. The levels of mRNA expression were normalized to GAPDH mRNA expression and expressed relative to the vehicle-treated cells. Values are expressed as means ± SE (n = 6 in each experiment). Insets show representative agarose gel electrophoresis of the amplified PCR products (23 cycles) from 0.1 μg of total RNA. B: immunoblot analysis using antibodies against AdipoR1 protein expression in cultured cardiomyocytes after incubation for 24 h with TNF-α (10 ng/ml), Norepi (100 nM), or vehicle in the presence or absence of Prava. The intensity of the β-tubulin band was used as a loading control between samples. The protein levels are expressed relative to the vehicle-treated cells. Values are expressed as means ± SE (n = 8–11 in each experiment). *P < 0.05.
Effect of pravastatin treatment on serum lipid, glucose, and insulin levels in mice. Pravastatin treatment for 4 wk had no effect on fasting serum levels of total cholesterol, triglycerides, glucose, and insulin, as shown in Table 2.

Effect of pravastatin on AdipoR1 expression in cultured cardiomyocytes. We previously reported that both TNF-α and norepinephrine inhibited mRNA expression levels of AdipoR1 in cultured cardiomyocytes (6). Thus we examined the effects of pravastatin on TNF-α- or norepinephrine-induced inhibition of AdipoR1 expression in cultured cardiomyocytes in the present study. Incubation with TNF-α or norepinephrine significantly inhibited expression levels of AdipoR1 mRNA and protein in cultured cardiomyocytes that was reversed by coincubation with pravastatin (1–10 μM) (Fig. 2). The effect of pravastatin to reverse TNF-α-induced inhibition of AdipoR1 expression was attenuated by coincubation with mevalonate (200 μM) (Fig. 3A). NAC also reversed TNF-α-induced inhibition of AdipoR1 expression (Fig. 3A). Incubation with H2O2 inhibited AdipoR1 mRNA expression that was also reversed by coincubation with pravastatin or NAC (Fig. 3B).

Role of adiponectin and AdipoR1 in glucose uptake and fatty acid oxidation of cultured cardiomyocytes. Insulin increased 2-deoxy-D-[3H]glucose uptake in cultured cardiomyocytes (Fig. 4A). Glb-adiponectin further increased insulin-stimulated glucose uptake in cultured cardiomyocytes (Fig. 4A). Pravastatin alone did not affect insulin-stimulated glucose uptake. Coincubation with TNF-α reduced the glb-adiponectin-induced increase in the glucose uptake. Coincubation with pravastatin reversed the reduction in the glb-adiponectin-induced increase in the glucose uptake in the presence of TNF-α (Fig. 4A). Transfection of siRNA specific for AdipoR1 attenuated the ability of pravastatin to reverse the TNF-α-induced reduction of the glucose uptake, whereas siRNA specific for AdipoR2 had no significant effect (Fig. 4A). In parallel, coincubation with pravastatin reversed the reduction in glb-adiponectin-induced increase in fatty acid oxidation in the presence of TNF-α, which was reversed by transfection of siRNA for AdipoR1 (Fig. 4B). The transfection of these siRNAs suppressed protein expression levels of the respective AdipoR1 and AdipoR2 (Table 3).

Effects of pravastatin on intracellular ROS production in cultured cardiomyocytes. Incubation of cultured cardiomyocytes with TNF-α increased DCF fluorescence intensity, which was reversed by coincubation with pravastatin or NAC (Figs. 5 and 6). Glb-adiponectin (0.25 μg/ml) had no effect. Coincubation with mevalonate attenuated the ability of pravastatin to reverse the TNF-α-induced increase in DCF fluorescence intensity (Figs. 5 and 6).

DISCUSSION

The present study demonstrated that AdipoR1 expression levels were decreased in the remote as well as the infarcted area of the left ventricle after MI and that oral administration of pravastatin reversed the decrease in AdipoR1 expression levels in the remote area independently of changes in serum cholesterol and insulin levels. Furthermore, with the use of cultured cardiomyocytes, coincubation with pravastatin also reversed the suppression of AdipoR1 expression after incubation with TNF-α or norepinephrine, potential mediators of postinfarction ventricular remodeling and dysfunction. Moreover, the present study showed that pravastatin reversed the reduction in the glb-adiponectin-induced increase in glucose uptake in the presence of TNF-α in cultured cardiomyocytes. The reversal by pravastatin of TNF-α-induced suppression of AdipoR1 expression might account for the improvement of glucose uptake, because inhibition of AdipoR1 expression with siRNA attenuated the effect of pravastatin. Therefore, it is
possible that pravastatin treatment might reverse the suppression of AdipoR1 expression in the infarcted heart, leading to improvement of glucose metabolism.

The present study showed that incubation with H2O2 suppressed AdipoR1 expression in cultured cardiomyocytes. Moreover, the suppression of AdipoR1 expression after incubation with TNF-α was reversed by NAC, a potent antioxidant. Pravastatin inhibited the TNF-α-induced production of intracellular ROS in cultured cardiomyocytes. Taken together, these results indicate that TNF-α suppressed AdipoR1 expression in cardiomyocytes at least partly through a mechanism of oxidative stress and that an antioxidative effect of pravastatin accounted for the attenuation of TNF-α-induced suppression of AdipoR1 expression. It is likely that the antioxidative effect of pravastatin depends on HMG-CoA reductase inhibition, because of the inhibitory effect of coinoculation with mevalonate.

This effect may be mediated by downstream isoprenoids in the mevalonate cholesterol pathway (i.e., geranylgeranylpophosphate, farnesylpyrophosphate, and squalene), as suggested in previous reports (25). AdipoR1 expression in cultured cardiomyocytes was not changed by incubation with pravastatin at lower concentrations of pravastatin (<1 μM) similar to the serum concentrations in humans or in mice (data not shown). In consideration of the fact that mice were treated with pravastatin over a period of 4 wk, this exposure time was much longer compared with the in vitro experiment where the cultured cardiomyocytes were treated with pravastatin only for 12–24 h. However, it was impossible to prolong the incubation time for 4 wk in the present in vitro experiment.

It is known that myocardial insulin resistance is associated with myocardial dysfunction (5, 13, 15, 20). A recent experi-

Table 3. Effect of siRNAs transfection on protein expression levels of AdipoRs

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<th>AdipoR1 Expression</th>
<th>AdipoR2 Expression</th>
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<tr>
<td>Control</td>
<td>1.00±0.02</td>
<td>1.00±0.09</td>
</tr>
<tr>
<td>Unrelated siRNA</td>
<td>0.89±0.09</td>
<td>0.93±0.06</td>
</tr>
<tr>
<td>AdipoR1 siRNA</td>
<td>0.38±0.02*</td>
<td>0.96±0.03</td>
</tr>
<tr>
<td>AdipoR2 siRNA</td>
<td>0.97±0.09</td>
<td>0.38±0.01*</td>
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Values are expressed as means ± SE; n = 6 in each experiment. After cells were washed, the extracts of the cells treated with each of the 3 siRNAs were used for the immunoblot analysis. The intensity of the β-tubulin band was used as a loading control between samples. The protein levels are expressed relative to the vehicle-treated cells (=1). *P < 0.05 vs. unrelated siRNA.
mental study (29) demonstrated that the restoration of myocardial insulin sensitivity in the failing heart protects from myocardial ischemia-reperfusion injury and improves myocardial function. Thus myocardial insulin resistance may be a target for the treatment of ischemic heart disease and heart failure (21). In this context, it is expected that a drug that increases myocardial expression of AdipoR1 could possibly improve myocardial insulin sensitivity and may be useful in the treatment of ischemic heart disease and heart failure (9).

It is known that oxidative stress and TNF-α are pathologically linked together with both insulin resistance and postinfarction myocardial dysfunction (2, 4, 24). Furthermore, several reports (2–4, 9, 12) have shown that oxidative stress and TNF-α counteract the insulin-sensitizing effect of adiponectin in adipose tissue. Moreover, it has been shown that oxidative stress suppresses adiponectin production and that adiponectin in turn exerts an antioxidative effect (7, 8, 23, 28). Therefore, adiponectin and its receptor system may have an important protective role against oxidative stress- or TNF-α-mediated myocardial insulin resistance and dysfunction after MI.

It is has been reported that adiponectin has protective actions against TNF-α-mediated myocardial injury and apoptosis in animal models of MI (19). We have previously shown that statin treatment increases circulating levels of adiponectin in patients with CAD in association with an improvement of endothelial function and the homeostasis model assessment for insulin resistance, a parameter of whole body insulin sensitivity (14). The present study showed that pravastatin reversed TNF-α-induced reduction in fatty acid oxidation as well as glucose uptake, both of which are essential for maintaining cardiac function (20). Therefore, the increase in myocardial AdipoR1 expression and circulating adiponectin levels may represent an important and additional mechanism for the beneficial effects of statins in ischemic heart disease and heart failure. However, it is known that the high rate of fatty acid oxidation suppresses glucose oxidation in the heart (20). In agreement with this effect, a recent study (16) showed that adiponectin had time-dependent effects on glucose metabolism in cultured neonatal rat cardiomyocytes, i.e., the initial increase and subsequent suppression after a 48-h incubation with adiponectin, which were accompanied by the increase in fatty acid oxidation. Although the long-term consequences of alteration of myocardial substrate selection in chronic ischemic heart disease are poorly understood, further studies are required for clarification of the possible role of the adiponectin-induced alteration of myocardial substrate selection in the pathophysiology of ischemic heart disease. Serum levels of glb-adiponectin are <1% of the total adiponectin levels (10–30 μg/ml), according to a previous study (11). Therefore, the dose (0.25 μg/ml) of glb-adiponectin used in the present study seems to be the same range of the serum concentrations.

In conclusion, pravastatin reversed the reduction of AdipoR1 expression in postinfarction mouse myocardium and in TNF-α-treated cardiomyocytes partly through an antioxidative mechanism in association with improvement of glucose uptake.

ACKNOWLEDGMENTS

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GRANTS

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