Asian Journal of Chemistry Vol. 21, No. 7 (2009), 5685-5693

Positive Inotropic Effect of Rosiglitazone in Papillary Muscles in Control and Diabetic Rats

SERVET KAVAK*, MUSTAFA EMRE†, ABDI BOZKURT‡ and HALIT DEMIR§
Department of Biophysics, Medical Faculty, Yüzüncü Yil University, Van, Turkey
Fax: (90)(432)2167519; Tel: (90)(432)2150470/1226
E-mail: skavak@cu.edu.tr; skavak@yyu.edu.tr

Peroxisome proliferator-activated receptor γ activators or rosiglitazone (RSG) used as insulin sensitizers in the treatment of diabetes. The aim of this study is the effects of RSG on papillary muscle positive inotropic were studied in left ventricular papillary muscles from both control rats and rats diabetes. In this study, we used four groups: (1) untreated control (C) (2) rosiglitazone-treated control (C + RSG), (3) diabetes (D) and (4) rosiglitazone-treated diabetes groups (D + RSG). Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) for 8 weeks STZ-treatment (STZ, 45 mg/kg). Papillary muscle from spontaneously diabetic hearts exhibited a depressed conraction force (CF), prolonged contraction time (CT) and half relaxation time (1/2 RT) and reduced contraction and relaxation velocities (± dp/dt) ($p < 0.05$). It is found that the lipid profile and glycohemoglobin levels (HbA1c) levels in D + RSG group as it increase the C rats value at the end of the treatment period ($p < 0.05$). D + RSG and C + RSG groups exhibited a increased CF, shortened CT and 1/2 RT and increased ± dp/dt ($p < 0.05$). Treatment of rats with RSG also markedly decreased the insulin resistance of the hearts. Present data suggest that the beneficial effects of RSG treatment on the mechanical activities of the diabetic rat papillary appear to be due to the restoration of the diminished SR Ca^{2+} release triggering, partially, related to the restoration of the hyperglycemia.

Key Words: Diabetes mellitus, Rosiglitazone, Papillary muscle, Excitation-coupling.

INTRODUCTION

Prevalence of cardiovascular disease is several fold high in patients with diabetes mellitus (DM). The most important cardiovascular complications of type 2 DM are diabetic cardiomyopathy characterized by an early diastolic and later systolic dysfunctions[1], microangiopathy, hypertrophy of cardiac myocytes[2] and finally heart failure[3]. The mechanisms of diabetic cardiomyopathy are still not fully clarified.

†Department of Biophysics, Medical Faculty, Çukurova University, Adana, Turkey.
‡Department of Cardiology, Medical Faculty, Çukurova University, Adana, Turkey.
§Department of Chemstry (Biochemistry), Art and Science Faculty, Yüzüncü Yil University, Van, Turkey.

Nevertheless, same studies showed that metabolic disturbances (depletion of glucose transporter 4 (GLUT 4), increased serum free fatty acids, carnitine deficiency, changes in calcium homeostasis), myocardial fibrosis (association with increased in angiotensin II, insulin-like growth factor I (IGF-I) and inflammatory cytokines), small vessel disease (microangiopathy, impaired coronary flow reserve and endothelial dysfunction), cardiac autonomic neuropathy (denervation and alterations in myocardial catecholamine levels) and insulin resistance[4]. They effect on development of severe diabetic cardiomyopathy.

Myocardial contractility is decreased in rats treated with STZ. It was found related to significant alterations of myocardial calcium metabolism[5]. Rosiglitazone, a thiazolidinedione, enhances peripheral insulin sensitivity in type 2 DM[6,7]. Effect of rosiglitazone on smooth muscle has been studied previously[8]. However there is no data about the effects of rosiglitazone on cardiac muscle.

The aim of this study is to investigate the effects of rosiglitazone on lipid profile and contractile activities in left ventricular papillary muscle in both healthy and diabetic rats.

EXPERIMENTAL

Animals handling and treatment protocol: Thirty two Wistar albino male rats (250-320 g and 8 weeks) were selected for the study and randomly divided into four equal groups. Animal care and experiments conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication NO.: 85-23, revised 1996) and approval of the ethics committee of our institution was obtained before the commencement of the study. Four groups were formed: (1) healthy control (C) (2) rosiglitazone-treated control, (C + RSG) (3) diabetes mellitus (D) and (4) rosiglitazone-treated diabetes mellitus groups (D + RSG). Diabetes mellutus was induced by a single intraperitoneal injection of streptozotocin (Sigma-Aldrich, USA; 45 mg/kg body weight). Streptozotocin was dissolved in 0.01 M sodium citrate, pH adjusted to 4.5. Animals were separated by 8 rats per cage and fed with standard rat nutrient and water without restriction throughout the experiment. Diabetic animals were randomly divided in two groups. One group of diabetic rats had been fed with standard rat nutrient for 8 weeks, the other group was treated with 4 mg/kg rosiglitazone two times a day by gavage. Starting from one week after the STZ injection treated-control rats were treated with 4 mg/kg rosiglitazone two times in a day by gavage as well[9].

Biochemical analysis

Measurements of HbA1c: Blood plasma HbA1c is determined immunoturbidimetrically by using Cobas Integra 800 (Roche Diagnostics, GmbH, Mannheim, Germany).

Measurement of lipids: Triglycerides (TG), total cholesterol (TC) and high density lipoprotein-cholesterol (HDL-C) were analyzed by GPO/PAP enzymatic colorimetric, CHOD/PAP enzymatic colorimetric and direct COHD/PAP enzymatic

colorimetric methods, respectively. The VLDL-C and LDL-C was calculated according to the equation described by Friedewald *et al.*[10]. All these parameters were determined by Cobas Integra 800 (Hitachi Modular Systems) biochemical analyzer (Roche Diagnostics, GmbH, Mannheim, Germany).

Isolation of left ventricular papillary muscles and contraction experiments: Wistar albino rats were anesthetized with ether and the hearts were quickly excised. The papillary muscles were dissected from the left ventricle and placed in the experimental chamber. The muscle was mounted in a party cup (about 2 mL volume) and perfuse continuously with oxygenated (95 % O_2 and 5 % CO_2) Krebs, (constituents in mmol/L: 113 NaCl, 4.7 KCl, 1.2 $MgSO_4 \cdot 7H_2O$, 1.9 $CaCl_2 \cdot 2H_2O$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 11.5 glucose, pH 7.4) solution at a constant flow.

The papillary muscle strips were suspended in organ baths containing Krebs solution, with a gas mixture of 95 % O_2 and 5 % CO_2 at 30 ºC and pH 7.35-7.45. After determining the thermoregulation and optimum muscle length the muscles were subjected to direct supramaximal stimulation with 0.1 Hz frequency square pulses for periods of 0.5 msec to obtain control values. [Nihon kohden Stimulator, FT.03 force displacement transducer and Hitachi Digital Storage Oscilloscope (VC-6045)].

The muscle contraction force (CF, mg), contraction and half-relaxation times (CT, 1/2RT, msec) and the contraction and relaxation rates (± dP/dt, g/msec) were determined[11] (Fig. 1).

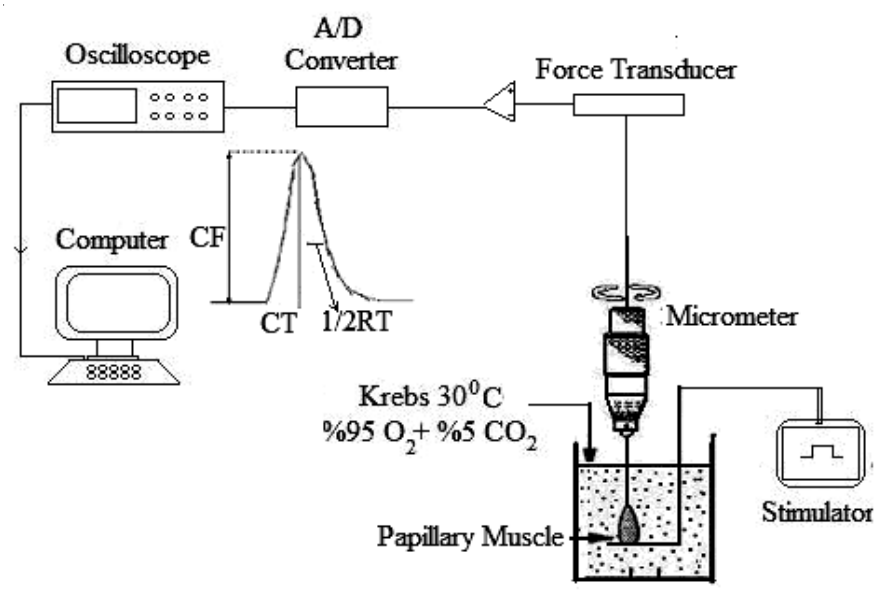

Fig. 1. The isolated organ bath and recorders. Twitchtension of the isolated rat papillary muscle and contraction parameters. CF: Contraction force, CT: Contraction time and 1/2 RT-Half relaxation times

Statistical analysis: Statistical analysis was performed by using SPSS 11.5.1 software (Lead Technologies, Inc., USA). All data represent as means ± standard error (SEM) of n observations. For all experiments, statistical analysis was performed by one way ANOVA followed by *post-hoc* analysis with the Bonferroni test to detect differences between control and experimental groups. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Glycohemoglobin levels and lipid profiles: The mean glycohemoglobin (HbA1c) level was lower in the D + RSG group than the D group. Rosiglitazone had significant effects on lipid profiles in the D + RSG group. The mean total cholesterol, LDL cholesterol, VLDL, triglyceride and glycohemoglobin (HbA1c) levels were higher in the D group than the controls, at the end of the study (Figs. 2 and 3).

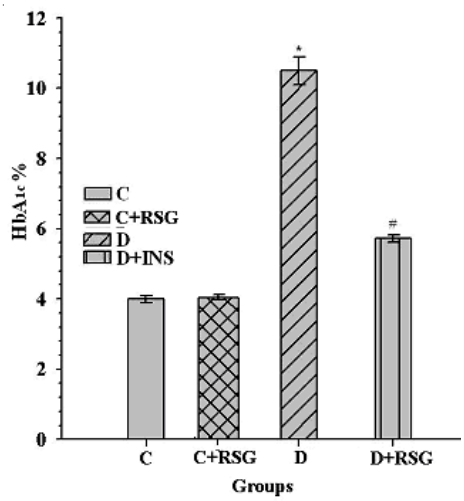

Fig. 2. Data are expressed as mean ± SEM. #Significant difference between D and D + RSG groups. *Significant difference between D and C groups

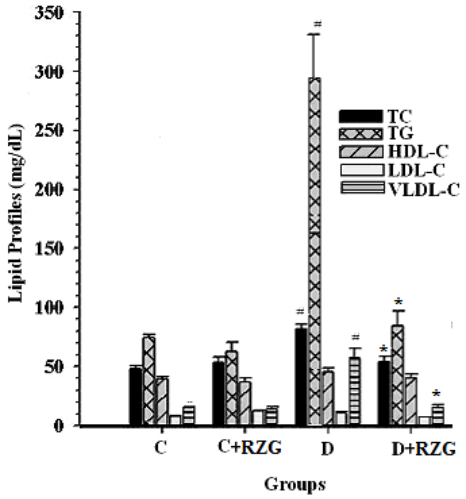

Fig. 3. Data are expressed as mean ± SEM. *Significant difference between D and D + RSG groups. #Significant difference between D and C groups

Effect of rosiglitazone on the contraction force parameters in left ventricular papillary muscles: The mean contraction force was found to be decreased in diabetic rats compared to the C group ($p < 0.05$). The use of rosiglitazon for 8th weeks resulted in increase in contraction force in both the diabetics and the controls (Fig. 4).

Effect of rosiglitazone on contraction and relaxation rates of papillary muscle: Papillary muscle from diabetic animals exhibited a reduced rates of contraction (+dp/dt) and relaxation (-dp/dt) ($p < 0.05$). In D + RSG group, the mean rates of conraction and relaxation increased compared to the D group ($p < 0.05$). However, contraction rates of the C + RSG and C groups were similar. The mean

relaxation rates of C + RSG was increased than the C group at the end of the study ($p < 0.005$) (Figs. 5 and 6).

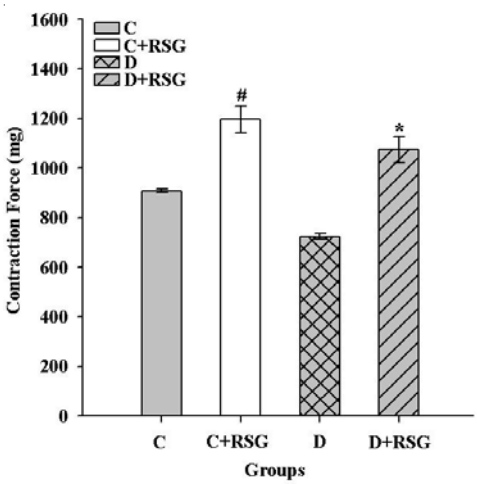

Fig. 4. Papillary muscle contraction force of the groups. Data are presented as mean ± SD. *Significant difference between D and D + RSG groups. #Significant difference between C and C + RSG groups

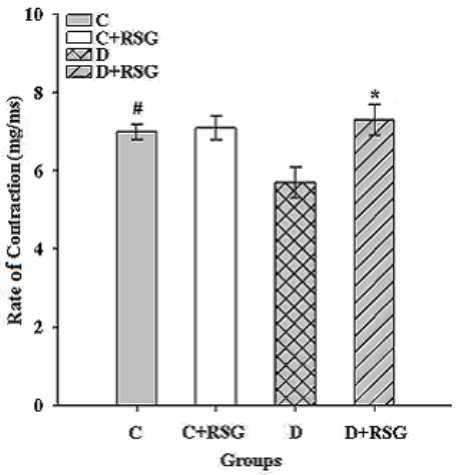

Fig. 5. Effect of rosiglitazone on contraction rate in papillary muscle. Data are presented as mean ± SD. *Significant difference between D and D + RSG groups. #Significant difference between C and C + RSG groups

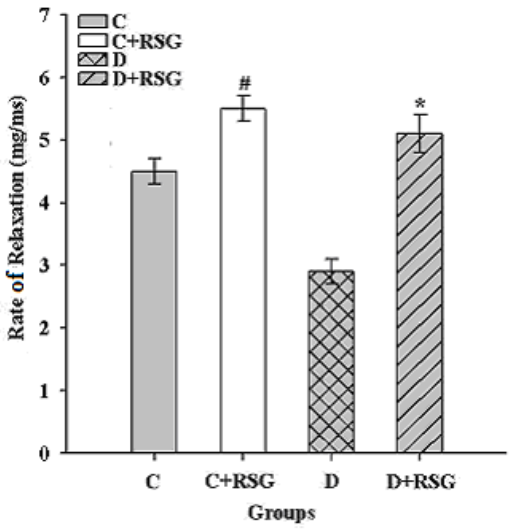

Fig .6. Effect of rosiglitazone on relaxation rate in papillary muscle. Data are presented as mean ± SD. *Significant difference between D and D + RSG groups. #Significant difference between C and C + RSG groups

Effect of rosiglitazone on papillary muscle contraction and half relaxation times: The mean contraction time decreased in the diabetic rats when compared to the control rats ($p < 0.05$). The effects of rosiglitazone on contraction time was insignificant in both diabetic and control rats ($p > 0.05$) (Fig. 7).The mean half relaxation time was found to be increased in diabetic rats. Rosiglitazone caused a significantly increasing in 1\2 RT in the controls, whereas shortening in diabetic rats (Fig. 8).

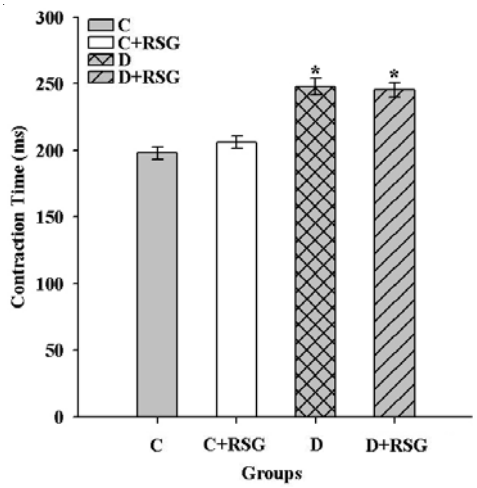

Fig. 7. Effect of rosiglitazone on contraction time in papillary muscle. Data are presented as mean ± SD. *Significant difference between D and D + RSG groups. #Significant difference between C and C+RSG groups

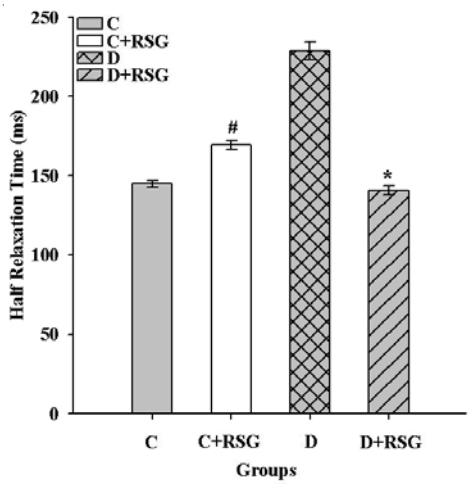

Fig. 8. Effect of rosiglitazone on half relaxation time in papillary muscle. Data are presented as mean ± SD. *Significant difference between D and D+RSG groups. #Significant difference between C and C+RSG groups

The main purpose of this study is to examine the effects of RSG on papillary heart muscle biomechanical parameters in a diabetic animal model by using the isole organ bath technique.

The main finding of the present study is that rosiglitazone exerted a significant positive inotropic effect in papillary muscles of both healthy control rats and rats with diabetes. RSG is an oral anti-diabetic agent used in type 2 diabetes mellitus and increases insulin sensitivity[12]. Insulin exerts Ca^{2+}-dependent and -independent positive inotropic effects through a phosphatidylinositol-3-kinase-dependent pathway in failing myocardium. The increased $[Ca^{2+}]_i$ originates at least in part from enhanced reverse-mode Na^+/Ca^{2+} exchange and consequently increased SR-Ca^{2+} load. In addition, rosiglitazone induced a positive inotropic effect without affecting the intracellular Ca^{2+} concentration. These results suggest that the Ca^{2+}-sensitizing effect is involved in the positive inotropic effect of rosiglitazone.

However, insulin exerts Ca^{2+}-dependent and -independent positive inotropic effects through a phosphatidylinositol-3-kinase-dependent pathway in failing human

myocardium. The increased $[Ca^{2+}]_i$ originates at least in part from enhanced reverse-mode Na^+/Ca^{2+} exchange and consequently increased SR-Ca^{2+} load. These nongenomic functional effects of insulin may be of clinical relevance, *e.g.*, during insulin-glucose-potassium infusions[13].

In previous studies[14,15], it is reported that rosiglitazone therapy improved HDL-C, TG, TC and LDL-C and decreased HDL-C levels. In a study patients who were receiving 8 mg rosiglitazone, exhibited no decrease in TG or LDL-C levels and a littel increase in HDL-C[16]. It was reported that 12 week rosiglitazone therapy caused a 13 % increase in HDL-C and a 19 % decrease in TG levels[8].

It is indicated that the effects of treatment with RSG on the body weight in diabetic rats are non-significant effects, but treatment of diabetic rats with rosiglitazone (3 mg/kg/day) showed the significant increase when compared with diabetic rats at the end of the treatment period[17]. In present study, it is found that the body weight and BGL in D+RSG rats were statistically increased when compared to the D rats[9].

There are deficient findings about effects of rosiglitazone on LDL-C and TC levels. Boyle *et al.*[18] found that rosiglitazone reduced triglycerides, but increased total cholesterol, LDL cholesterol and reduced HDL cholesterol. In contrast, pioglitazone reduced triglycerides, total cholesterol, LDL cholesterol and increased HDL-C. Conversely, in their study, Myerson *et al.*[19] observed reduced plasma fatty acid concentrations and hepatic triglyceride content after rosiglitazone therapy.

It is found that the TG, TC, HDL-C, LDL-C and VLDL-C levels in D + RSG rats as it reached the C rats value at the end of the treatment period.

In present study, STZ-treated rats developed diabetes with all characteristic clinical symptoms *i.e.* increased blood glucose and decreased body weight[9]. Eight weeks lasting diabetes led in present conditions to a considerable reduction of cardiac contraction force. In addition to it, both contraction and relaxation rate were slowed down in diabetes. These findings are similar to that with earlier studies[18,19]. With regard to basic mechanisms of the negative inotropic effect of diabetes a considerable amount of work was done on cellular and molecular level. A number of diabetes-induced changes was found on the level of Ca^{2+} handling: reduced IcaL[17], decreased number of SR ryanodine receptors[20], decreased mRNA and protein levels of SERCA2[5,21,22], further inhibition of SERCA2 by increased level of unphosphorylated phospholamban[5], decreased mRNA and protein levels of Na^+/Ca^{2+} exchanger[21,23,24]. Significant alterations were also described for contractile proteins: diminished Ca^{2+} sensitivity, shifts in myosin isoenzymes (from V1 myosin with high ATPase activity to V3 myosin with low ATPase)[25]. The main finding of the present study is that rosiglitazone exerted a significant positive inotropic effect in papillary muscles of healthy control rats treated with rosiglitazone and diabetic rats treated with rosiglitazone. The data indicate that the positive inotropic effect of rosiglitazone is related to ICaL and processes of triggering SR Ca^{2+} release (processes of SR Ca^{2+} loading, namely SR Ca^{2+} pump).

In case of RSG treated diabetes, present study revealed that the contraction time (CT) and relaxation (1/2 RT) was shortened in sustained spontaneous diabetes, associated with to speed up contraction and relaxation rate (± dp/dt). Probably, effects of RSG on contraction and relaxation mechanisms. The exact mechanism of the inotropic effect of rosiglitazone remains unclear. Recently, new inotropic agents that increase Ca^{2+} sensitivity have been produced and have attracted much attention. Such drugs are called Ca^{2+}-sensitizers' and are considered useful because no fear of Ca^{2+} overload which induces ventricular arrhythmia, tachycardia and increasing myocardial oxygen consumption leading to myocardial damage[26]. In the present study, it is demonstrated that rosiglitazone exerts its inotropic effect without affecting intracellular Ca^{2+} handling or increasing intracellular Ca^{2+} concentration. These observations suggest that rosiglitazone may exert its positive inotropic effect through a Ca^{2+}-sensitizing effect. It has been reported that the Ca^{2+}-sensitizing effect is mediated by (1) an enhancement of the affinity between Ca^{2+} and troponin C, (2) an acceleration of the interaction among troponin, tropomyosin and actin or (3) facilitation of the formation of cross bridges[26]. Regarding the biomechanic effects of rosiglitazone, however, there are some differences between rosiglitazone and Ca^{2+}-sensitizers. For example, Ca^{2+}-sensitizers reduce the diastolic function by increasing Ca^{2+} sensitivity even in the diastolic phase[10].

In conclusion, rosiglitazone increases inotropy in rat cardiac papillary muscle. This leads to an increase in the Ca^{2+} influx into the cardiac cells, which improves mechanical performance

ACKNOWLEDGEMENT

This work was supported by the Çukurova University Research Projects Department (No. TF2006D3).

REFERENCES

1. M.A. Maghoub and A.S. Abd-Elfattah, *Mol. Cell. Biochem.*, **180**, 59 (1998).
2. P. Gargiulo, G. Jacobellis, V. Vaccari and D. Andreani, *Diabetes Nutr. Metab.*, **11**, 336 (1998).
3. R.J. Jarret, *Diabet. Metab. Rev.*, **5**, 547 (1989).
4. Z.Y. Fang, J.B. Prins and T.H. Marwick, *Endocr. Rev.*, **25**, 543 (2004).
5. R.A. Brown, M.J. Anthony, P. Petrovski and J. Ren, *Cardiovasc. Toxicol.*, **1**, 35 (2001).
6. J.M. Lehmann, L.B. Moore, T.A. Smith-Oliver, W.O. Wilkison, T.M. Willson and S.A. Kliewer, *J. Biol. Chem.*, **270**, 12953 (1995).
7. A.J. Wagstaff and K.L. Goa, *Drugs*, **62**, 1805 (2002).
8. C. Schindler, C.M. Ferrario, C. Jatzke, K. Ahner, K.B. Brosnihan, P. Bramlage, S.M. Seber, S. Ucak, O. Basat and Y. Altuntas, *Diabetes Res. Clin. Pract.*, **71**, 52 (2006).
9. S. Kavak, M. Emre, T. Tetiker, T. Kavak, Z. Kolcu and I. Günay, *Arch. Pharmacol.*, **376**, 415 (2008).
10. W.T. Friedewald, R.I. Levy and D.S. Fredrickson, *Clin. Chem.*, **18**, (1972)
11. M. Emre, I. Karayaylali, M. San, A. Demirkazik and S. Kavak, *Arch. Pharm. Res.*, **27**, 646 (2004).
12. H.S. Ahn, S.E. Kim, H.J. Jang, M.J. Kim, D.J. Rhie, S.H. Yoon, Y.H. Jo, M.S. Kim, K.W. Sung, S.Y. Kim and S.J. Hahn, *Naunyn Schmiedebergs Arch Pharmacol.*, **374**, 305 (2006).

13. D. Von Lewinski, S. Bruns, S. Walther, H. Kögler and B. Pieske, *Circulation*, **24**, 111, 2588 (2005).
14. L. Olansky, A. Marchetti and H. Lau, *Clin. Ther. Suppl.*, **25B**, 64 (2005).
15. J.P. Van Wijk, E.J. Koning, E.P. Martens and T.J. Rabelink, *Arterioscler Thromb. Vasc. Biol.*, **23**, 1744 (2003).
16. A.B. King, *Diabetes Care*, **23**, 557 (2000).
17. J. Chaudhry, N.N. Ghosh, K. Roy and R. Chandra, *Life Sci.*, **80**, 1135 (2007).
18. P.J. Boyle, A.B. King, L. Olansky, A. Marchetti, H. Lau and R. Magar, *Clin. Ther.*, **24**, 1358 (2002).
19. A.B. Myerson, R.S. Hundal, S. Dufour, V. Lebon, D. Befory and G.W. Cline, *Diabetes*, **51**, 797 (2002).
20. J. Ren, M.F. Walsh, J.R. Sowers and R.A. Brown, *Life Sci.*, **65**, 369 (1999).
21. H.W. Kim, Y.S. Cho, R.H. Lee, S.Y. Park and Y.H. Kim, *Life Sci.*, **70**, 367 (2001).
22. Z. Yu, G.F. Tibbits and J.H. Mcneill, *Am. J. Physiol.*, **266**, H2082 (1994).
23. J.C. Lee and S.E. Downing, *Am. J. Physiol.*, **230**, 1360 (1976).
24. Y. Hattori, N. Matsuda, J. Kimura, T. Ishitani, A. Tamada, S. Gando, O. Kemmotsu and M. Kanno, *J. Physiol.*, **527**, 85 (2000).
25. A. Malhotra and V. Sanghi, *Cardiovasc Res.*, **34**, 34 (1997).
26. M. Endoh, *Gen. Pharmacol.*, **26**, 1 (1995).

(*Received*: 11 December 2008; *Accepted*: 30 April 2009) AJC-7523