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Modulation of AT-1R/AMPK-MAPK cascade plays crucial role for the pathogenesis of diabetic cardiomyopathy in transgenic type 2 diabetic (Spontaneous Diabetic Torii) rats

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ABSTRACT

There are evidences that the activation of AMPK is playing pivotal role in the lipid and glucose metabolism. It has been reported that both the AMPK and angiotensin-II acts as a negative regulator for each protein. It has been well proven that the MAPK cascade could be modulated by the presence of angiotensin-II. Moreover, studies were shown that p38 MAPK stimulates glucose uptake through the AMPK activation. Therefore, we speculate and tried to demonstrate that the modulation of AT-R/MAPK pathway through AMPK might play crucial roles for the pathogenesis of diabetic cardiomyopathy, using the transgenic (Spontaneous Diabetic Torii – SDT) rats. We performed Western blot analysis for the measurement of myocardial AT-R, AMPK and MAPK cascades-related protein expressions, p67-phox and caspase-12. In addition, we employed dihydroethidium (DHE), Azan Mallory and hemotoxylin eosin (HE) staining methods to demonstrate the superoxide radical production, fibrosis and hypertrophy, respectively. The protein expressions, such as AT-1R, p-ERK1/2, p67-phox and caspase-12 were found to be significantly increased and conversely, the Ang-(1-7) *mas* R, Tak1, LKB1 and p-AMPKα1, p-p38 MAPK and p-JNK protein expressions were found to be considerably decreased in the SDT rats, in comparison to the normal rats. The DHE, Azan Mallory and HE stainings also revealed that the SDT rats have more superoxide radical production, fibrosis and hypertrophy, respectively than the normal rats. Taken together, it is suggested that the modulation of AT-1R/AMPK-MAPK pathway might play crucial roles for the pathogenesis of diabetic cardiomyopathy and it could become an important therapeutic target to ameliorate the diabetic cardiomyopathy.

1. Introduction

AMP-activated protein kinase (AMPK) is a phylogenitically conserved fuel-sensing enzyme that is present in both primitive unicellular organisms and mammals and is a serine–threonine kinase, which has emerged as a crucial regulator of diverse cellular pathways, especially energy balance in mammalian cells [1,2]. Energy deprivation associated with hypoxia and ischemia, exercise,

Abbreviations: ACE-2, angiotensin converting enzyme-2; AMPK, AMP-activated protein kinase; Ang-(1-7) *mas* R, angiotensin-(1-7) *mas* receptor; Ang-II, angiotensin-II; AT-1R, Ang-II type 1 receptor; CVP, central venous pressure; DHE, dihydroethidium; ±*dp*/d*t*, rate of intra-ventricular pressure rise and decline; EF, ejection fraction; ERK1/2, extracellular signal regulated kinase 1/2; FS, fractional shortening; HE, hemotoxylin eosin; IVSd, intra-ventricular septum thickness in diastole; IR, insulin resistance; JNK, c-Jun-N-terminal kinase; LKB1, serine/threonine kinase 11; LVDd, left ventricular dimension in diastole; LVDs, left ventricular dimension in systole; LVEDP, left ventricular end-diastole pressure; LVP, left ventricular pressure; LVPWd, left ventricular posterior wall thickness in diastole; MAPK, mitogen activated protein kinase; mmHg, millimeter mercury; mmHg/s, millimeter mercury/second; p38 MAPK, p38 mitogen activated protein kinase; p-AMPKα1, phospho-AMP-activated protein kinase alpha1; p-ERK1/2, phospho- extracellular signal regulated kinase 1/2; p-JNK, phospho-c-Jun-N-terminal kinase; p-p38 MAPK, phospho-p38 mitogen activated protein kinase; RAS, rennin-angiotensin system; SD, Sprague–Dawley; SDT, Spontaneous Diabetic Torii; STZ, streptozotocin; Tak1, transforming growth factor-beta activated kinase 1.

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and pressure overload leads to the activation of AMP-activated protein kinase (AMPK) in the heart [3]. AMPK is activated in response to the increase in the cellular AMP/ATP ratio [4,5], and then switches on ATP-generating pathways and switches off ATP-consuming pathways. A large number of studies have highlighted the importance of the activation of AMPK for the amelioration of diabetic-related consequences on the myocardial cells and to maintain the cardiac physiology under high glucose condition [6], but, whether the activation or attenuation of AMPK provides beneficial effects to cardiac physiology and pathology is still controversial [6]. Furthermore, interestingly, recent reports are coming out with the inter-relation between two stress-response pathways, such as AMPK and mitogen-activated protein kinases (MAPKs). MAPKs include three major subfamilies, such as extracellular-signal regulated kinases (ERKs), c-Jun-N-terminal kinase (JNK) and p38 MAPK [7]. It has been reported that MAPKs play dominant role in diverse cellular responses, includes cell survival, growth and differentiation and apoptosis [8]. Previously, it has been believed that the ERKs was shown to regulate cell proliferation and differentiation, while p38 MAPK and JNK were shown to mediate cellular stress and apoptosis [9].

Moreover, it has been reported that both the AMPK and Ang-II plays negative regulator for each protein. Recently, Schuhmacher et al. [27,28] have reported that the activation of AMPK during Ang-II treatment improves the endothelial function by inhibiting NADPH oxidase and xanthine oxidase, and Nox2 up-regulation. These results clearly indicate the link between Ang-II and AMPK. We, recently have reported that the modulation of AT-1R/MAPK cascade by an AT-1R receptor blocker, olmesartan could play a significant role in the attenuation of diabetic nephropathy in hyperglycemic condition in mice. In addition, recent studies have reported that the MAPK cascade is involving in the glucose metabolism, especially the p38 MAPK has been reported to be involved in the glucose uptake through AMPK, and the curcumin and berberine have been reported to stimulate the glucose uptake through AMPK-p38 MAPK in myotubes [10,11], but the exact role of other MAPKs, such as ERK and JNK is undefined clearly.

Nowadays, studies have shown that MAPK cascade has had a close relationship in glucose metabolism. Furthermore, MAPK cascade was proven to be involved in the pathogenesis of cardiac hypertrophy in streptozotocin (STZ)-induced diabetic condition [12]. Moreover, the p38 MAPK has been reported to play a biphasic role, such as increased glucose uptake and development of cardiac hypertrophy under high glucose condition. So, therefore, we thought and tried to demonstrate that the modulation of AT-R/MAPK cascade through AMPK might play an interesting role for the pathogenesis of diabetic cardiomyopathy using novel spontaneous diabetic rats.

2. Materials and methods

2.1. Materials

Dihydroethidium (DHE) was purchased from Molecular Probes, Eugene, OR, USA. Phosphatase arrest-III was purchased G-Biosciences, St. Louis, MO, USA. Aprotinin, leupeptin and sodium dodecyl sulfate were purchased from Sigma–Aldrich, Tokyo, Japan. Trizma base, sodium chloride, sodium fluoride, sodium orthovanadate, 2-mercaptoethanol, glycerol, bromophenol blue, bovine serum albumin (BSA) and Tween 20 were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan.

2.2. Experimental design

Eight weeks old male SDT rats (CLEA Japan, Tokyo, Japan) were used for the study. Age-matched Sprague–Dawley (SD) rats (Charles River Japan, Kanagawa, Japan) were used as control animals. The animals were maintained in controlled room (temperature 23 ± 2 °C, humidity $55 \pm 15\%$, 12 h lighting cycle). The rats were divided into two groups SD, ($n = 8$); and Spontaneous Diabetic Torii (SDT, $n = 8$) and they were allowed free access to water and chow throughout the period of study (32 weeks) and they were treated in accordance with the guidelines from animal experimentation of our institute and Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

2.3. Assessment of myocardial functions by hemodynamic and echocardiographic studies

At the end of study (week 32), the rats were anesthetized with 2% halothane in oxygen during the surgical procedures for the measurement of hemodynamic parameters. A catheter-tip transducer (Miller SPR 249; Miller Instruments, Houston, TX) was introduced into the left ventricle (LV) through the right carotid artery to determine the peak left ventricular pressure (LVP), LV end-diastolic pressure (LVEDP), central venous pressure (CVP) and the rates of intraventricular pressure rise ($+dp/dt$) and decline ($-dp/dt$) were recorded as described previously [13]. After instrumentation, the concentration of halothane was reduced to 0.5% to minimize the effects of the anesthetic on hemodynamic parameters. Echocardiographic studies were carried out with a 7.5-MHz transducer (Aloka Inc., Tokyo, Japan). The LV dimensions in diastole (LVDd) and systole (LVDs), percent fractional shortening (FS) and ejection fraction (ES), intra-ventricular septum thickness in diastole (IVSd), LV posterior wall thickness in diastole (LVPWd) were estimated using the M-mode measurements.

2.4. Biochemical estimation

The blood was collected from the tail vein at different period of time in the tube containing the heparin. The collected blood was centrifuged at 3500 rpm for 15 min at 4 °C for the separation of plasma and it was stored at −80 °C until the assays were performed. The plasma was used for the estimation of insulin. The measurement of blood glucose (BG) was done at every week with the Nipro Freestyle Freedom (Nipro, Osaka, Japan).

2.5. Histopathological study

Finally, rats were sacrificed and the hearts were excised. The excised heart tissues were cut into 2-mm-thick transverse slices and fixed in 10% formalin and unfixed for the frozen section. After being embedded in paraffin, several transverse sections were obtained from heart and stained with hemotoxylin and eosin (HE). Also, the samples were stained with Azan-Mallory to demonstrate fibrosis in the heart. The measurement for fibrosis and hypertrophy were assessed semi-quantitatively as previously reported [14].

2.6. In situ detection of superoxide radical production in the heart

To evaluate in situ superoxide radical production from the heart, unfixed frozen cross section of the specimens were stained with dihydroethidium according to the previously validated method [15].

2.7. Western blotting

The ventricular protein lysate was prepared from the myocardium as described previously [15]. The total protein concentration in samples was measured by the bicinchoninic acid (BCA) method. For the determination of protein levels, equal amounts of protein extracts (50 μg) were separated by 7.5–10% SDS polyacrylamide gel electrophoresis (Bio-Rad, CA, USA) and

transferred electrophoretically to nitrocellulose membranes. Membranes were blocked with 5% non-fat dry milk or BSA in Tris buffered saline Tween (20 mM Tris, pH 7.6, 137 mM NaCl, and 0.1% Tween 20). Primary antibodies against Tak1, LKB1, p-AMPKα1, and angiotensin-II type 1 receptor (AT-1R), were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Primary antibody against Ang-(1-7) *mas* R was obtained from Alomone Labs Ltd., Jerusalem, Israel. Primary antibodies against p38 MAPK, p-p38 MAPK, ERK1/2, p-ERK1/2, JNK, p-JNK and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were obtained from Cell Signaling Technology Inc., Beverly, MA, USA. Primary antibody against caspase-12 was obtained from Biovision Research Products, CA, USA.

All the antibodies used at a dilution of 1:1000. The membrane was incubated overnight at 4 °C with the primary antibody, and the bound antibody was visualized using the respective horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology Inc.) and chemiluminescence developing agents (Amersham Biosciences, Buckinghamshire, UK). The levels of GAPDH, p38 MAPK (for p-p38 MAPK), ERK1/2 (for p-ERK 1/2), and JNK (for p-JNK) were estimated in every sample to check for equal loading of samples. Films were scanned, and band densities were quantified with densitometric analysis using Scion Image program (Epson GT-X700, Tokyo, Japan). All values were normalized by setting the density of normal samples as 1.0.

2.8. Statistical analysis

Data are represented as means ± standard error of mean (S.E.M.). Statistical analysis of differences between groups was performed by student's *t* test using GraphPad Prism 5.0 software. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Levels of blood glucose and plasma insulin in the SD and SDT rats

The delayed onset of DM associated with the hypoinsulin secretion is the hallmark for the SDT rats. The measurement of BG and plasma insulin levels revealed that the SDT rats were shown significant increase in the BG from the age of week 16 and peaked at week 20 and maintained the similar pattern of BG level throughout the period of study. In addition, the measurement of plasma insulin also revealed that the SDT rats were of less in the plasma insulin significantly throughout the period of study (Fig. 1A and B).

3.2. Myocardial function in the SD and SDT rats

The measurement of CVP, LVP, LVEDP and ±d*p*/d*t* by hemodynamic method gives an indication for the proper functioning of the heart. In this study, the value for CVP was shown significant increase in the SDT rats than in the SD rats and the value for LVP and ±d*p*/d*t* were shown significant decrease in the SDT rats in comparison to the SD rats. Interestingly the LVEDP value seems to increase in the SDT rats but statistically not significant, which clearly indicates the systolic and diastolic dysfunction of the heart in the SDT rats. Furthermore, echocardiographic analysis also shown that the LVDd and LVDs were considerably increased and the percent EF and FS were significantly decreased in the SDT rats, in comparison to the SD rats. Unfortunately, the IVSd and LVPWd showed no significant changes between the groups. These findings clearly indicate that the SDT rats have profound myocardial dysfunction than the SD rats (Table 1 and Fig. 1C and D).

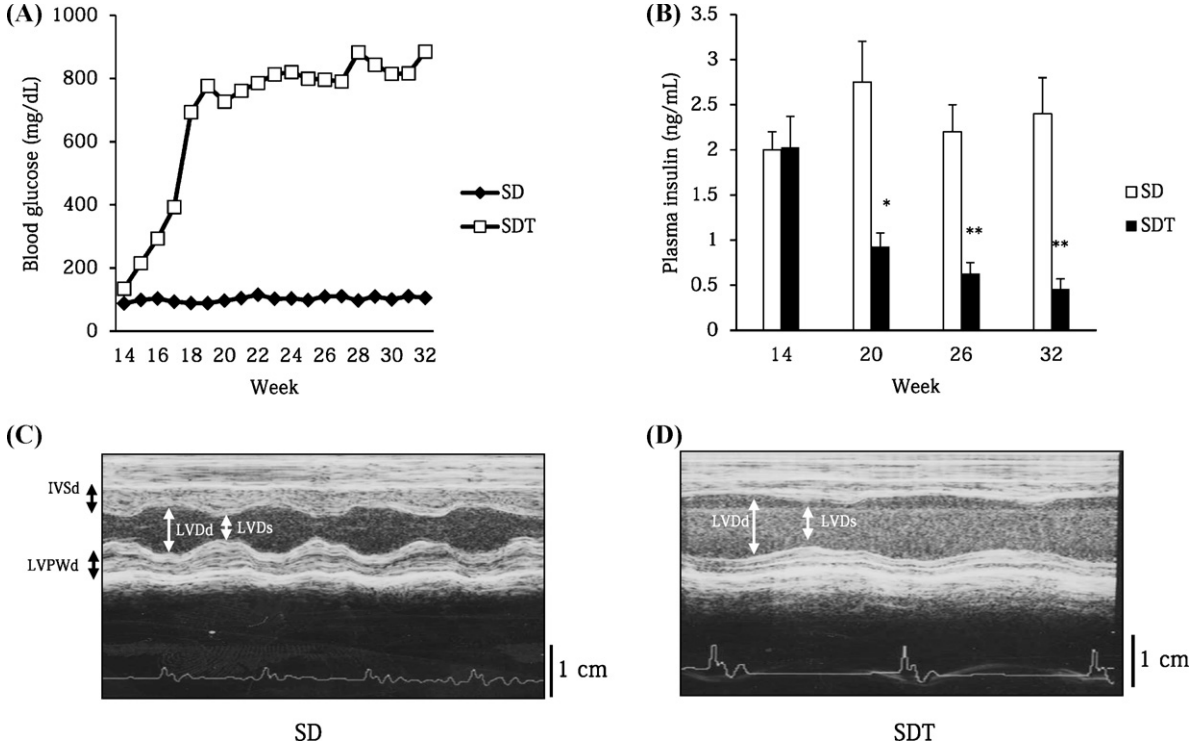

Fig. 1. (A and B) showing the BG and plasma insulin levels for the SD and SDT rats. (C and D) showing echocardiographic image for the SD and SDT rats. LVDd, left ventricular dimension in diastole; LVDs, left ventricular dimension in systole; IVSd, intra-ventricular septum thickness in diastole; LVPWd, left ventricular posterior wall thickness in diastole. Scale bar represents 1 cm. SD, age-matched Sprague–Dawley rats; SDT, age-matched Spontaneous Diabetic Torii rats. All values are expressed as the mean ± S.E.M.; $N = 6$; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs SD.

Table 1
Comparison of hemodynamic and echocardiographic parameters between the SD and SDT rats.

	SD ($n=6$)	SDT ($n=6$)
Hemodynamic data		
CVP (mmHg)	0.57 ± 0.08	$2.55 \pm 0.28^{**}$
LVP (mmHg)	121.8 ± 8.3	$84.94 \pm 5.40^{**}$
+dp/dt (mmHg/s)	7174 ± 364	$3131.5 \pm 265.5^{**}$
−dp/dt (mmHg/s)	7616 ± 412	$3166.0 \pm 475.0^{**}$
LVEDP	13.13 ± 2.67	28.62 ± 8.00
Echocardiographic data		
LVDd (mm)	8.40 ± 0.48	$9.82 \pm 0.17^{*}$
LVDs (mm)	4.65 ± 0.38	$6.52 \pm 0.34^{**}$
FS (%)	44.68 ± 1.98	$32.83 \pm 2.58^{*}$
EF (%)	80.68 ± 1.95	$65.93 \pm 3.49^{*}$
IVSd (mm)	2.15 ± 0.08	2.18 ± 0.05
LVPWd (mm)	2.18 ± 0.04	2.27 ± 0.011

Results are presented as the mean ± SEM. n, no. of rats. CVP, central venous pressure; LVP, left ventricular pressure; {±} dp/dt, rate of intra-ventricular pressure rise and decline; LVEDP, left ventricular end-diastolic pressure; LVDd, left ventricular dimension in diastole; LVDs, left ventricular dimension in systole; FS, fractional shortening; EF, ejection fraction; IVSd, intra-ventricular septum thickness in diastole; LVPWd, left ventricular posterior wall thickness in diastole; SD, age-matched Sprague–Dawley rats, SDT, age-matched Spontaneous Diabetic Torii rats.
$^{*}P < 0.05$ vs SD. $^{**}P < 0.01$ vs SD.

3.3. Expression of NADPH oxidase subunit and superoxide radical production in the SDT rats

Since, oxidative stress has been the significant part in many of the Ang-II mediated deleterious effects on myocardium, especially in hyperglycemic conditions; we measured the protein expression of NADPH oxidase subunit, p67-phox in both the SDT and SD rats. Expectedly, the p67-phox protein expression was significantly enhanced in the SDT rats, in comparison to the SD rats (Fig. 2A). Furthermore, induction of oxidative stress leads to the more production of superoxide radical. So, we employed DHE staining to measure the superoxide radical production in both the SDT and SD rats. The production of superoxide radicals were found to be higher in the SDT rats, in comparison to the SD rats (Fig. 4A), which confirms the induction of oxidative stress in the SDT rats.

3.4. Modulation of the renin-angiotensin system (RAS) components in the SDT rats

It has been proven that the RAS components could play the important roles in the pathogenesis of type 2 diabetes mellitus (DM). So, we performed Western blot analysis to measure the protein expressions of RAS components, such as AT-1R and Ang-(1-7) *mas* R. We found that the protein expression of AT-1R was significantly up-regulated in the SDT rats, when compared with the SD rats (Fig. 2B). Interestingly, we found that the Ang-(1-7) *mas* R protein expression was significantly down-regulated in the SDT

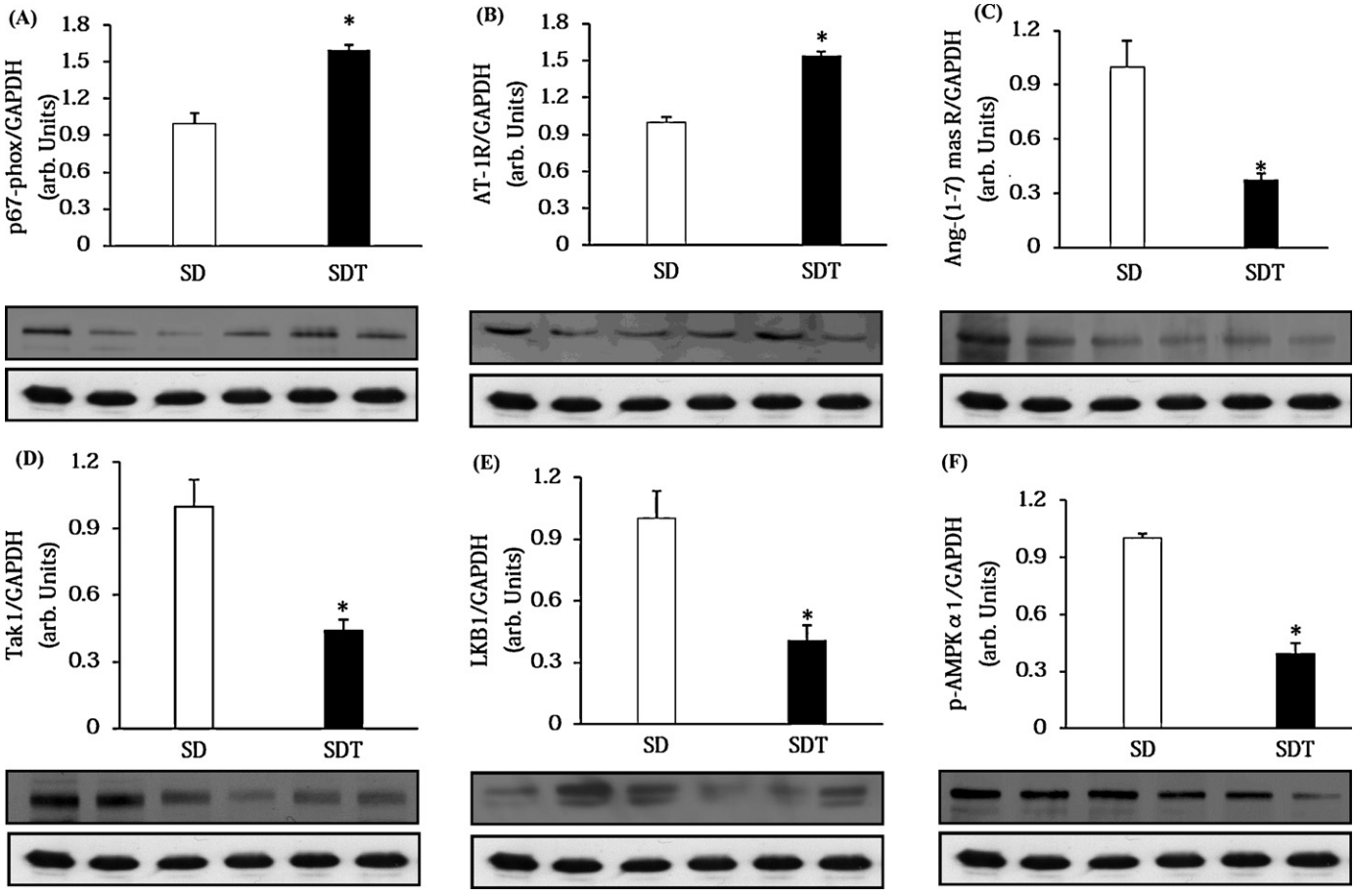

Fig. 2. Protein expressions of NADPH oxidase subunit p67-phox, RAS components, and AMPK and its upstream kinases in both the SD and SDT rats. Representative Western blots (lower panel) show specific bands for (A) p67-phox, (B) AT-1R, (C) Ang-(1-7) *mas* R, (D) Tak1, (E) LKB1 and (F) p-AMPKα1 and the representative histograms (upper panel) show the band densities of 5–6 samples compared with relative GAPDH band density and expressed in arbitrary units. An equal amount of protein sample obtained from the myocardial homogenate was applied to each lane. SD, age-matched Sprague–Dawley rats; SDT, age-matched Spontaneous Diabetic Torii rats. All values are expressed as the mean ± S.E.M.; $N = 5–6$; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs SD.

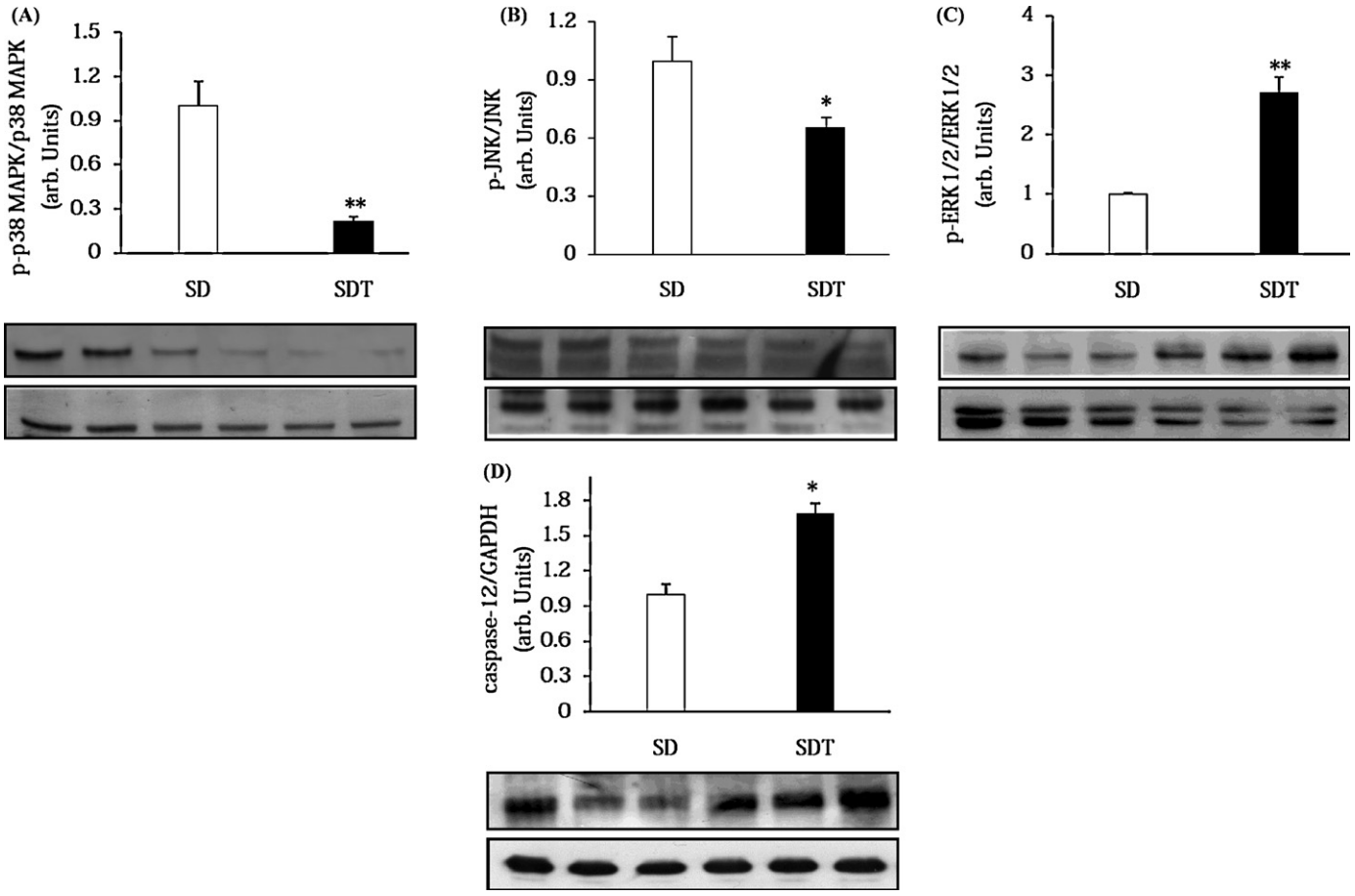

Fig. 3. Protein expressions of MAPK cascade and caspase-12 in both the SD and SDT rats. Representative Western blots (lower panel) show specific bands for (A) p-p38 MAPK, (B) p-JNK, (C) p-ERK1/2, and (D) caspase-12 and the representative histograms (upper panel) show the band densities of 5-6 samples compared with relative p38 MAPK (for p-p38 MAPK), JNK (for p-JNK), ERK1/2 (for p-ERK1/2), and GAPDH (for caspase-12) band densities and expressed in arbitrary units. An equal amount of protein sample obtained from myocardial homogenate was applied to each lane. SD, age-matched Sprague–Dawley rats; SDT, age-matched Spontaneous Diabetic Torii rats. All values are expressed as the mean ± S.E.M.; $N = 5–6$; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs SD.

rats, in comparison to the SD rats (Fig. 2C). Convincingly, these results indicate that the SDT rats were shown significant modulation of the RAS components, which might be linked with further deleterious consequences on the SDT rats.

3.5. Protein expressions of AMPK and its upstream kinases in the SDT rats

The hyperglycemia plays main role in the pathogenesis of type 2 DM in the SDT rats. Since, AMPK has been central regulator for glucose and fatty acid metabolism in mammalian cells; so therefore, we measured the protein expressions of AMPK and its upstream kinases, such as Tak1 and LKB1. Interestingly, we have found that the protein expressions of Tak1, LKB1 and p-AMPKα1, were significantly down-regulated in the SDT rats, when compared with the SD rats (Fig. 2D–F).

3.6. Differential protein expressions of MAPK cascade and caspase-12 in the SDT rats

Recently, it has been reported that the AMPK-mediated glucose uptake was increased via the enhanced phosphorylation of p38 MAPK. This leads us to measure the protein expressions of MAPK cascade in both the SDT and SD rats. Interestingly, we have found that the SD rats were shown significant up-regulation in the phosphorylation of p38 MAPK and JNK, in comparison to the SD rats (Fig. 3A and B). In addition, increased protein expression of p-ERK1/2 in the SDT rats in comparison to the SD rats also revealed that the MAPK cascade is expressed differentially in the SDT rats (Fig. 3C). Furthermore, caspase-12 protein expression was also increased in the SDT rats than the SD rats (Fig. 3D).

3.7. The SDT rats showed significant increase in myocardial hypertrophy and fibrosis

It has been reported that the activation of myocardial MAPK cascade are closely associated with the hypertrophy, fibrosis and apoptosis. Azan Mallory and HE stainings were revealed that the myocardial hypertrophy and fibrosis were increased in the SDT rats, in compared with the SD rats (Fig. 4B and C).

4. Discussion

In this study, the SDT rats were shown statistically significant increase in the CVP, LVDd and LVDs than the normal rats. In addition, the LVEDP also increased but statistically not significant. Furthermore, the SDT rats were shown significant decrease in the LVP, $\pm dp/dt$, and percent FS and EF when compared with the normal rats. These results clearly indicate that the SDT rats have compromised myocardial function (Table 1).

Activation of AMPK has been demonstrated to play a pivotal role in maintaining the cardiac energy balance through the activation of an energy producing pathways and inhibition of an energy consuming pathways [16]. It has been reported that the

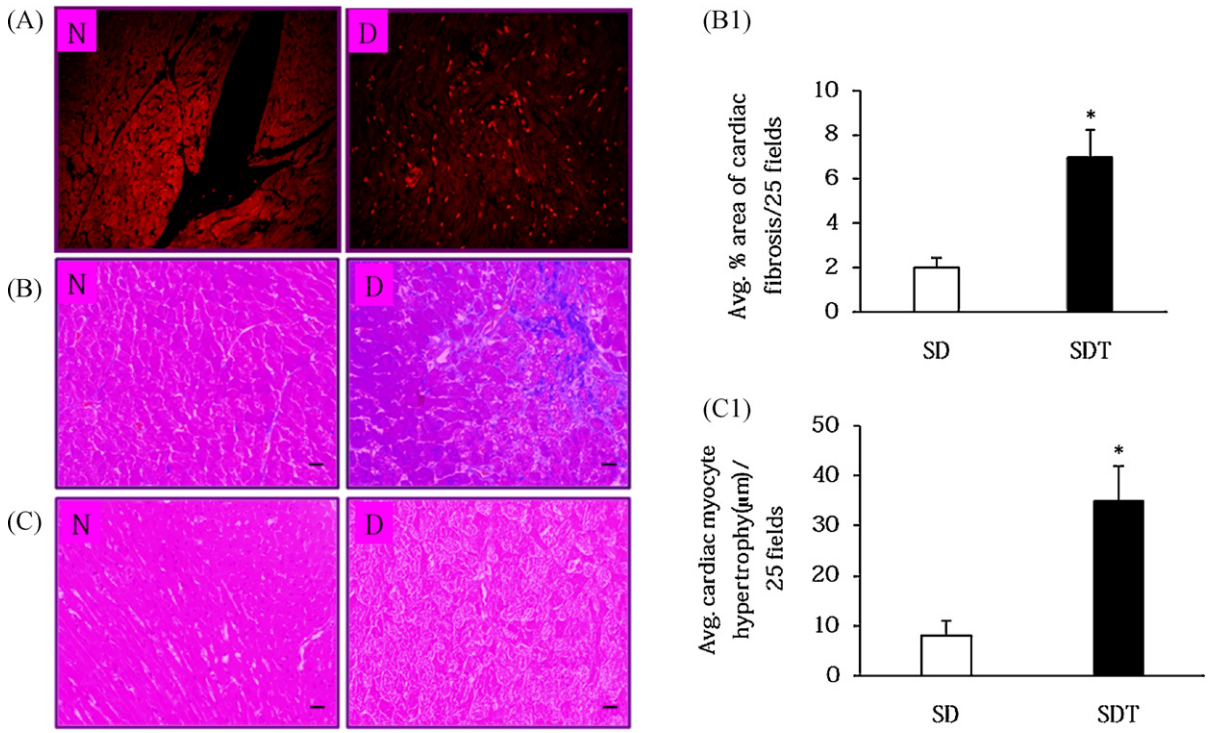

Fig. 4. DHE staining, Azan Mallory staining and HE staining for the measurement of myocardial superoxide radical production, fibrosis and hypertrophy in both the SD and SDT rats. (A) shows myocardial superoxide radical production [200×] in SD and SDT groups. (B and C) represents Azan-Mallory staining and HE staining for myocardial fibrosis and hypertrophy, respectively in group SD and SDT groups. Fibrosis is indicated by the blue area as opposed to red colour in myocardial cells (200×). (B1 and C1) shows the respective quantitative bar graphs for fibrosis and hypertrophy in SD and SDT groups. Scale bar represents 20 μm. SD, age-matched Sprague–Dawley rats; SDT, age-matched Spontaneous Diabetic Torii rats. All values are expressed as the mean ± S.E.M.; $N = 3$ per group. $^{*}p < 0.05$ vs SD. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

activation of AMPK enhances glucose uptake, utilization of fatty acid and decreases fatty acid synthesis, thereby involving in the enhancement of glucose and lipid metabolism as well as improvement in the insulin resistance (IR) [17,18]. In addition, the activation of myocardial AMPK is greatly suppressed by an excessive IR in diabetic condition [19]. It has been demonstrated that the Tak1 and LKB1 are proven as potential upstream kinases of AMPK [20]. Consistent with the previous reports, we also found that the SDT rats were of less in the p-AMPKα1, Tak1 and LKB1 protein expressions than the normal rats (Fig. 2D–F). Moreover, biochemical estimation also revealed that the SDT rats have had increased IR as well as exacerbated lipid accumulation, such as LDL, HDL, TG and TC than the normal rats (unpublished data). From these results, now, it is obvious that the SDT rats have had compromised myocardial energy balance which might be due to the reduced phosphorylation of AMPK.

Furthermore, studies are coming out with the crosstalk between the myocardial AMPK and RAS, which has provided an excellent opportunity for the researchers to know the precise mechanism for the progression of diabetic cardiomyopathy. It has been reported that the activation of AMPK potentially inhibits the Ang-II-stimulated vascular smooth cell proliferation, myocardial metabolic switch and hypertrophy [21,22] and improves the endothelial function via the inhibition of NADPH oxidase, xanthine oxidase and Nox-2 in the treatment of Ang-II [23,24]. These results provide novel information that the RAS components and AMPK has had a close relation and AMPK works as a negative feedback regulator role in Ang-II mediated deleterious effects on diabetic cardiomyopathy. In line with the previous reports, our study has shown that the superoxide radical production and protein expressions of p67phox, AT-1R were found to have increased (Fig. 2A and B and 4A) and interestingly protein expression of Ang-(1-7) *mas* R was decreased in the SDT rats (Fig. 2C), when compared with the SD rats. Since, we and others have reported that the Ang-(1-7) *mas* R has potentially improves the cardiac and renal function in diabetic condition [25–27], it seems that the Ang-(1-7) could provide beneficial effects on Ang-II mediated deleterious action via the activation of AMPK. Although, at this stage, it is premature to say and further researches are warranted on this aspect. Taken together, it is suggested that the SDT rats were shown significant enhancement of RAS components, which greatly affects the activation of myocardial AMPK via the enhancement of NADPH oxidase and superoxide radical production.

Moreover, it has been well proven that the activation of RAS components could render deleterious effects, such as hypertrophy and apoptosis in diabetic conditions through the stimulation of one of the potential stress response cascade, MAPK. We recently have reported that the modulation of AT-1R/MAPK cascade potentially involves for the pathogenesis of diabetic nephropathy in experimental animal model [25]. So, we speculate that the activation of Ang-II due to hyperglycemia might differentially regulate the MAPK cascade via AMPK-dependent mechanism in type 2 DM. It has been reported that the quercetin treatment could attenuates the obesity through the mediation of AMPK and MAPK signaling pathways [28]. Furthermore, curcumin and berberine have been shown to stimulate the glucose uptake through AMPK-p38 MAPK pathways in L6 myotube cells [11,29]. In line with the previous reports, we also have found the attenuated myocardial phosphorylation of p38 MAPK (Fig. 3A) and increased GLUT4 gene expression in the whole myocardial cell lysate (data not shown) in the SDT rats, in comparison to the normal rats. These results clearly suggests that the SDT rats have reduced glucose uptake due to the blunt in the phosphorylation of AMPK-p38 MAPK pathway which eventually results in an impairment of glucose metabolism leading to an excessive IR.

Furthermore, another potential entity of MAPK cascade is ERK. It has been reported that the activation of AMPK significantly inhibited the growth and proliferation in cardiac fibroblasts and apoptosis in HCT116 carcinoma through the inhibition of ERK [28,30]. Kim et al. (2001) have reported that AMPK differentially regulates ERK cascades by inhibiting the RAS activation or stimulating the RAS-independent mechanism [31]. From the previous reports, it is suggested that the activation of AMPK might antagonize the ERK-mediated myocardial effects and also it is largely depends on cell specific. In our study also, we found that the SDT rats have shown drastic increase in the expression of p-ERK1/2 protein, in comparison to normal rats (Fig. 3C). So, it is clearly described that the activation of myocardial AMPK could potentially antagonize the Ang-II stimulated ERK1/2 in non-obese type 2 diabetic condition.

We found another interesting result is that the SDT rats were shown significant decrease in the phosphorylation of JNK protein, in comparison to the SD rats (Fig. 3B). There have been a numerous studies which emphasized the role of JNK for the promotion of pro-apoptotic and attenuation of anti-apoptotic signaling cascades [32–34]. We recently have reported that the activation of JNK through AT-1R cascade in hyperglycemic condition promotes the renal apoptosis in mice [25]. Interestingly, it has been reported that the activation of AMPK promotes apoptosis on insulin producing MIN6 cells and autophagic cell death in chronic myelogenous leukemia cells through the activation of JNK [35,36]. Conversely, Kaiser et al. (2005) have reported that the acute inhibition or chronic activation of JNK protects the myocardium in vivo [37]. Moreover we have found an increased caspase-12 protein expression in the SDT rats than the SD rats (Fig. 3D). So, our finding supports the fact that the chronic inhibition of p-JNK might cause deleterious effects on myocardium through various mechanisms.

Eventhough we did not find the significant difference in IVSd and LVPWd, but HE staining revealed that the SDT rats were found to have increased cardiomyocyte hypertrophy in comparison to the normal rats (Fig. 4C). Moreover, the Azan Mallory staining revealed that the SDT rats were shown significant increase in the myocardial fibrosis, when compared with the SD rats (Fig. 4B). Recently, it has been reported that AMPK deficient mice are prone to develop the hepatic fibrosis [38] and AMPK activation by adiponectin ameliorated the hepatic fibrosis by the suppression of cytokine signaling-SOCS3 [39]. Furthermore, AMPK agonists prevented the cystic fibrosis in epithelial cells [40]. Taken together, it is suggested that the attenuation of myocardial AMPK due to the hyperglycemia mediated stimulation of Ang-II might enhance the cardiac hypertrophy and fibrosis in type 2 diabetic condition.

In conclusion, considering all these findings, it is suggested that the chronic activation of hyperglycemia-induced Ang-II might have differential consequences on MAPK cascade, such as stimulation of p-ERK1/2, and attenuation of p-p38 MAPK and p-JNK via the attenuation of master censor of energy cascade, AMPK. In addition, modulation of AT-1R/AMPK-MAPK cascade will eventually leads to the decreased glucose uptake as well as myocardial dysfunction due to the attenuated phosphorylation of AMPK in type 2 DM. Furthermore, the drugs which modulate the AT-1R/AMPK-MAPK cascade could become potential therapy for the amelioration of diabetic cardiomyopathy.

Conflict of interest

No conflict of interest.

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