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ORIGINAL RESEARCH

Advanced Glycation End Products Downregulate Connexin 43 and Connexin 40 in Diabetic Atrial Myocytes via the AMPK Pathway

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Purpose: Diabetes mellitus is an independent risk factor for atrial fibrillation (AF), which may be related to accumulation of advanced glycation end products (AGEs). However, the mechanisms involved are not completely clear. Abnormality of gap junction proteins, especially connexin 43 (Cx43) and connexin 40 (Cx40) in atrial myocytes, is an important cause of increased susceptibility of AF. The aim of our work is to investigate the mechanism of dysregulated Cx43 and Cx40 in atrial myocytes of diabetic rats.

Methods: We established a type 1 diabetic rat model by intraperitoneal injection of streptozotocin. HL-1 cells and primary rat atrial myocytes were treated with AGEs in vitro. Using Western blotting, immunofluorescence staining, immunohistochemistry, and lucifer yellow diffusion measurements, we investigated dysregulation of Cx43 and Cx40 and its mechanism in atrial myocytes of diabetic rats.

Results: Accumulation of AGEs was found in diabetic rats. The expression of Cx43 and Cx40 was reduced in the atrium of diabetic rats, accompanied by the decrease of phosphorylated Adenosine 5'-monophosphate-activated protein kinase (p-AMPK). Similar results were found in cultured HL-1 cells and primary rat atrial myocytes, suggesting a role of AGEs on gap junction proteins. An AMPK agonist, 5-Aminoimidazole-4-carboxamide ribonucleoside (AICAR), reversed the down-regulated Cx43 expression induced by AGEs stimulation. More importantly, lucifer yellow diffusion assay showed that AGEs significantly affected gap junctional function, and these changes were reversed by AICAR.

Conclusion: Thus, we conclude that AGEs cause dysregulation of Cx43 and Cx40 in diabetic atria via the AMPK pathway, thereby leading to gap junction dysfunction, which may contribute to the increased AF susceptibility in diabetes.

Keywords: diabetes, atrial fibrillation, advanced glycosylation end products, adenosine 5'-monophosphate-activated protein kinase, connexin

Introduction

Diabetes mellitus (DM) is a chronic progressive metabolic disease with a serious threat to human health. The International Diabetes Federation announced that the amount of diabetic patients has increased to 537 million worldwide in 2021. The number is still growing at an alarming rate globally and it seems to affect 693 million adults by 2045.[1] DM disrupts vital organs in the body and leads to various long-term complications, of which cardiovascular complications are major causes of increased morbidity and mortality, mainly including cardiac arrhythmias, heart failure, myocardial infarction and even sudden cardiac death.[2–4] It has been found that atrial fibrillation (AF) is an independently associated risk factor for individuals with DM.[5] DM plays an important role in structural remodeling, electrical remodeling, autonomic remodeling, connexin remodeling, and oxidative stress, thus promoting the occurrence of AF.[5] However, the specific pathophysiology of AF complicated by DM has not been fully elucidated.

Advanced glycation end products (AGEs) are the biochemical end products of non-enzymatic glycosylation reactions (Maillard reaction).[6] It is a stable covalent addition produced from the spontaneous reaction of macromolecules such as proteins, lipids or nucleic acids with glucose or other reducing monosaccharides.[6] AGEs have two sources in the body: the synthesis of AGEs in the body from excess sugar and protein, and the ingestion of AGEs present in food through diets. The production of AGEs is moderate under normal physiological conditions. However, it is significantly accelerated due to increased glucose utilization in the case of diabetes.[7] Thus, AGEs are considered to be a key factor in the progression of diabetic complications.[7–9] Recently, studies have reported that AGEs are related to arrhythmias both in patients[10] and animal models.[11] AGEs induced senescence of atrial myocytes and increase susceptibility of AF in diabetic mice via reversing electrical remodeling.[12] However, knowledge of the role of AGEs in DM-related AF remains limited.

Connexin is a membrane protein that is an important component for gap junction channels in the myocardium and responsible for the communication between neighboring cardiomyocytes, of which connexin 43 (Cx43) is the most abundant one in cardiomyocytes. Besides, Cx40 is selectively expressed in atrial myocytes and is mainly involved in coordinating the electrical activation of atria.[13] It has been shown that connexin plays a key role in propagating action potentials between cardiomyocytes. The pathogenesis of AF is significantly associated with slower conduction and reentry formation resulting from downregulation of Cx43, disruption of atrial gap junctions and enhanced fibrosis.[14] Cx40 has also been reported to be engaged in the onset and progression of AF to varying extents.[15] However, whether AGEs play a role in regulating gap junctional proteins is still unknown. Therefore, this study examined the role of AGEs in regulating gap junction proteins, which may underlie DM-related AF.

Materials and Methods
Experimental Animal Models

Type 1 diabetic animal models were established as previously reported.[16] Briefly, 8-week-old male Sprague-Dawley (SD) rats, weighing approximately 180 g (n = 28, purchased from Cavins Laboratory Animals, Changzhou, China) were injected intraperitoneally with streptozotocin (STZ, 60mg/kg, S0130, Sigma-Aldrich, St. Louis, MO, USA). One week after injection, blood was drawn from the tail veins and random blood glucose was measured using an Accu-Chek glucose meter (Roche Diagnostics GmbH Company, Germany). Rats with blood glucose concentration >16.7 mmol/L were enrolled in this study as the diabetic group, and vehicle-injected, age-matched rats served as controls. All rats were raised at a standard of care-specific pathogen-free (SPF) conditions (temperature 22–24°C and humidity 55–65%) in a light/dark cycle adapted to 12h. After 8 weeks, body weight and random blood glucose were measured. The rats were then sacrificed, and their hearts were harvested and stored at −80°C. All experiments were conducted according to the internationally accepted principles by the ethical guidelines by the International Council for Laboratory Animal Science (ICLAS) and all animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing Medical University.

Isolation of Neonatal Rat Atrial Myocytes

Primary rat atrial myocytes were extracted from the atrial tissue of neonatal rats using the cardiomyocyte isolation kit (88281Y, Pierce Thermo Fisher Inc., Rockford, IL, USA) according to manufacturer's instructions as previously reported.[12,17] The rat bodies were disinfected with 75% ethanol, and then the hearts were rapidly isolated and placed in 1.5 mL sterile tubes containing 500 µL of phosphate buffered saline. Atrium were cut into 1 mm^3 pieces and washed. An appropriate amount of Cardiomyocyte isolation enzyme 1 (88288E, Thermo Fisher) and cardiomyocyte isolation enzyme 2 (88289E, Thermo Fisher) was added into the shredded tissue. Then, we incubated tubes at 37°C. After 30 minutes, the tissues were washed twice with PBS. DMEM containing 10% fetal bovine serum (FBS) was added to each tube, mixed by pipetting, and then seeded and incubated in 1×10^6/100-mm Petri dishes. The unattached cells were seeded at 2×10^5/well in 6-well plates and initially incubated at 37°C and 5% CO_2 after 2 hours. Primary atrial myocytes were supplemented with fresh culture medium 2–3 days after isolation and treated in 5–7 days after isolation. To investigate the effect AGEs on Cx43 and Cx40 expression, primary atrial myocytes were exposed to 100 µg/mL AGEs-BSA

(ab51995, Abcam) for 24 hours. Cells cultured with 100 μg/mL non-glycated BSA (A3803, Sigma) were used as a control.

HL-1 Cell Culture

HL-1 cells (m077, iCell, Shanghai, China) were cultured in DMEM containing 10% FBS. When cell fusion reached approximately 70%, HL-1 cells were treated with AGEs-BSA or BSA similar as primary rat atrial myocytes. To observe whether Adenosine 5'-monophosphate-activated protein kinase (AMPK) agonist AICAR (2840, TOCRIS, Bristol, UK) could reverse the effect of AGEs, AGEs+AICAR group and BSA+AICAR group were set. Cells were first treated with AGEs for 24 hours then with 10 μmol/L AICAR for another 24 hours,[18,19] to test whether post-AGE AMPK activation leads to normalization of Cx43 and Cx40 expression.

siRNA Transfection

AMPK α1 siRNA (S: 5′-CUGUGGCUCACCCAAUUAUTT-3′, AS: 5′-AUAAUUGGGUGAGCCACAGTT-3′), AMPK α2 siRNA (S: 5′-GAGCGACUAUCAAAGACAUTT-3′, AS: 5′-AUGUCUUUGAUAGUCGCUCTT-3′) and negative control (NC) siRNA (S: 5′-UUCUCCGAACGUGUCACGUTT-3′, AS: 5′-ACGUGACACGUUCGGAGAATT-3′) were purchased from Gene Pharma (Shanghai, China). HL-1 cells were cultured into 6-well plates and transfected with siRNA (100 nmol/L final) for 8 hours using Lipofectamine 2000 liposome (11668019, Thermo Fisher, USA). The ratio of Lipofectamine 2000 over siRNA in a well was 10 μL: 10 μL. The siRNA-transfected cells were used for Western blotting analysis at 48–72h post infection.

Western Blot

Atrial tissues, HL-1, primary rat atrial myocytes were homogenized and lysed with ice-cold RIPA buffer containing protease and phosphatase inhibitors. Equal amounts of protein (30 μg) were separated on a 10% SDS-polyacrylamide gel and transferred to PVDF membranes. After blocking with 5% non-fat milk in tris-buffered saline containing Triton-X100, membranes were hybridized overnight with the specific primary antibodies AGE (1:1000, ab23722, Abcam), rabbit polyclonal to RAGE (1:1000, ab65965, Abcam), p-AMPK (1:1000, 2535S, Cell Signaling Technology), AMPK (1:1000, 2532S, Cell Signaling Technology), Cx43 (1:500, sc-365107, Santa Cruz Biotechnology, Dallas, Texas, USA) and Cx40 (1:500, sc-271837, Santa Cruz Biotechnology). Then, the membranes were incubated with horseradish peroxidase (HRP)-conjugated anti-rabbit IgG or anti-mouse IgG for 1.5 hours. Expression of β-tubulin (1:10000, ab21058, Abcam) was determined as a housekeeping control. After washing, membranes were developed with electrochemilumi-nescence (ECL) reagents (wbkls0500, Millipore) and images were taken by the ECL imager (Tanon-5200 Multi, Tanon Technology Co., Ltd). Quantification was performed with ImageJ.

Immunohistochemistry

Hearts were harvested and fixed in 4% paraformaldehyde and cut into 5 μm slices after embedding in paraffin after 8 weeks. Then, the sections were incubated with anti-Cx40 and anti-Cx43 antibodies overnight at 4°C. The next day, the primary antibody was eluted and the secondary antibody was incubated at 37° for 30 min, followed by incubation with SABC at 37° for 30 min, DAB development, and then counterstaining with hematoxylin. Following dehydration, coverslips were mounted with neutral balsam). Finally, the images were captured with a light microscope. The positive signals for Cx40 and Cx43 proteins are brown.

Lucifer Yellow Diffusion

Neonatal rat atrial myocytes cultured in 6-well plates were washed with PBS. After aspiration of the liquid, 0.05% fluorescent dye fluorescein yellow (L0259, Sigma) was added to the wells and immediately scratched with a scalpel blade.[20] Cells were rinsed with PBS three times and fixed with 4% paraformaldehyde. Images were observed under a fluorescence microscope (EVOS, Thermo Fisher).

Statistical Analysis

Data were presented as mean ± SEM. The normality of the distributions was analyzed by Shapiro Wilk test. Student's *t*-test was used to compare data between two groups if data normally distributed; otherwise, the Mann–Whitney *U*-test was used. One-way ANOVA was used to compare data from multiple groups if data normally distributed, and LSD or Tamhane's were adopt as post hoc tests; otherwise, the Kruskal–Wallis test followed by Dunn's was used. Statistical significance was defined as $P < 0.05$. Graphing and statistical analysis were performed with GraphPad Prism 8 and SPSS 27.0.

Results
Establishment of Type 1 Diabetes Model Induced by Streptozotocin

Eight weeks after STZ injection, DM rats showed significant increase in blood glucose and decrease in body weight, while control rats showed no significant changes in blood glucose but increase in body weight (Figure 1A and B). The protein levels of AGE and RAGE in the atrial tissue of two groups of rats were measured. As is shown in Figure 1C and D, the expressions of AGE and RAGE were significantly higher in the atria of diabetic rats compared to the normal group. This result indicates that diabetes increases the accumulation of AGEs.
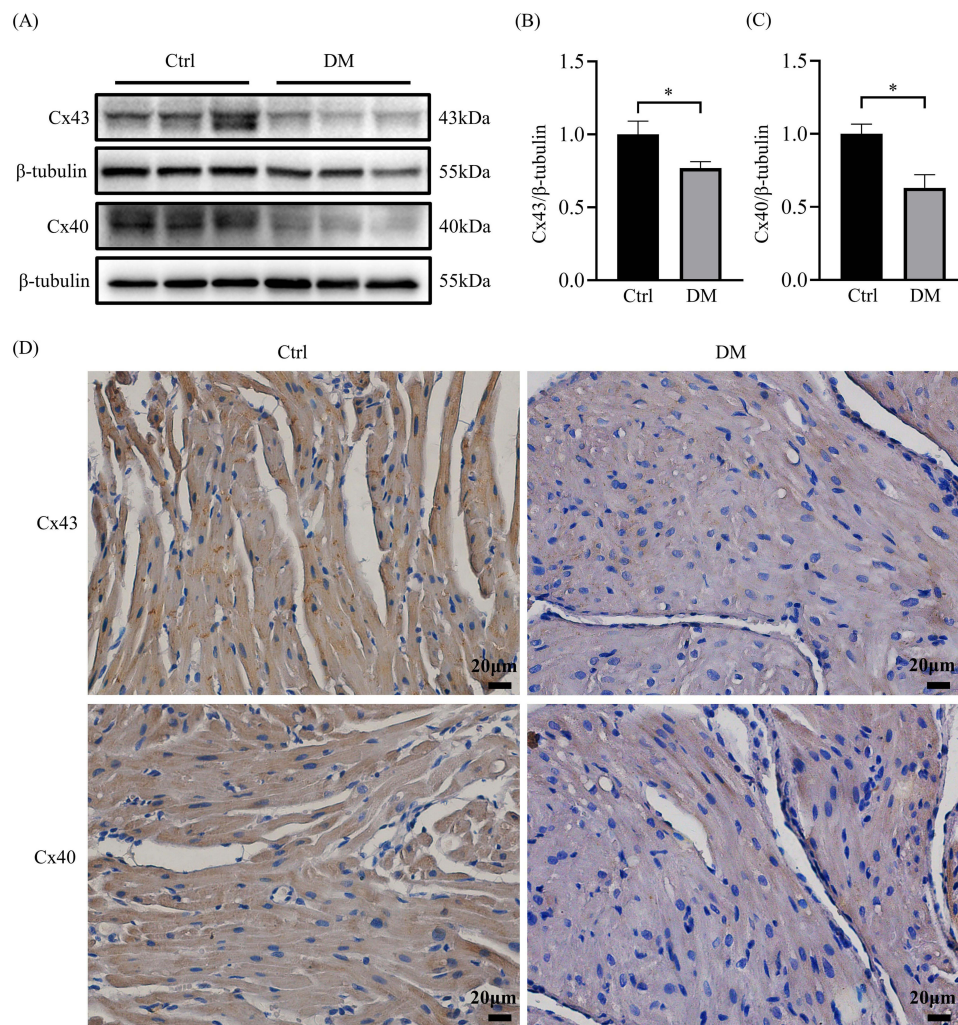
Expression of Cx43 and Cx40 in Myocardial Tissue of Diabetic Rats

The protein levels of Cx43 and Cx40 in the atrial tissue of two groups were analyzed by Western Blot. As is shown in Figure 2A-C, both Cx43 and Cx40 were significantly downregulated in the atria of diabetic rats compared to the control rats. Consistently, as assessed by immunohistochemistry, the expression of Cx43 and Cx40 was downregulated in DM group, and their distribution in the atria became disturbed (Figure 2D). These results suggest remodeling of connexins was detected in the atria of diabetic rats.
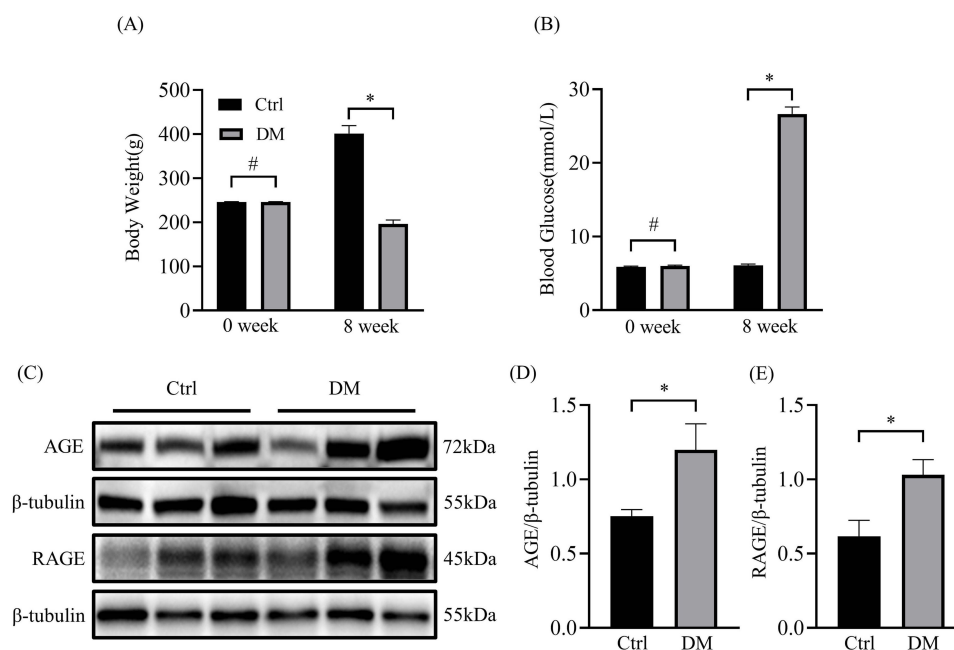
Figure 1 Body weight and blood glucose of diabetic rats and the expression of AGE and RAGE in the atrium. (**A**) Changes in body weight of two groups of rats in 0 week and 8 week (n=12). (**B**) Blood glucose changes in two groups of rats before and after modeling (n=12). (**C**) Representative blots of AGE and RAGE proteins in atrium of diabetic rat (n=6). (**D**) Densitometry analysis of AGE and RAGE proteins in atrium of diabetic rat (n=6). *p<0.05, #p>0.05.
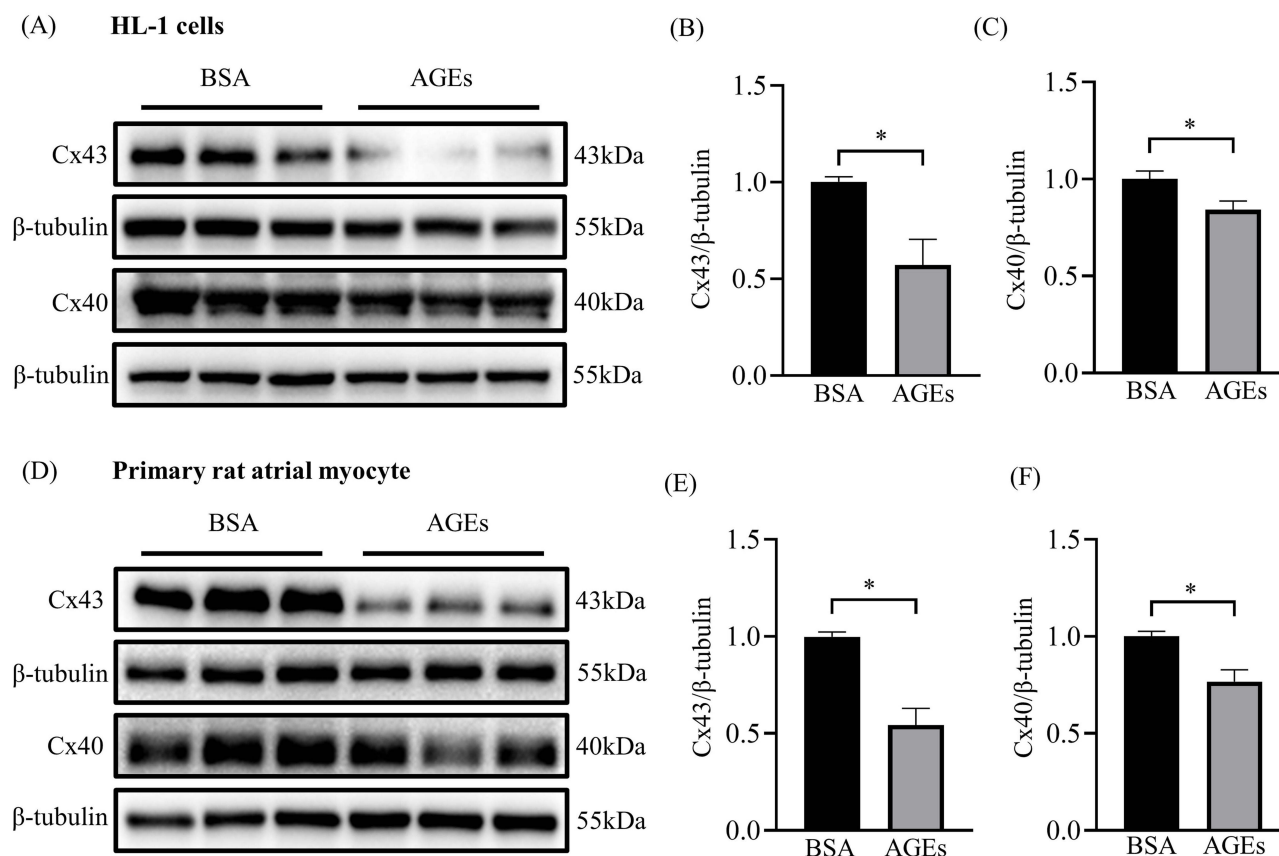
Figure 2 Expression of Cx43 and Cx40 proteins in atrial tissues from Control and Diabetic groups. (**A**) Representative blots of Cx43 and Cx40 proteins in atrium of diabetic rat (n=6). (**B** and **C**) Densitometry analysis of Cx43 and Cx40 proteins in atrium of diabetic rat (n=6). (**D**) Representative histological fixed sections of atrial tissues stained with Immunohistochemistry. *p<0.05.

Effects of AGEs on Cx43 and Cx40 in Cultured HL-1 and Primary Neonatal Rat Atrial Myocytes

To determine whether AGEs play a role on the regulation of Cx43 and Cx40 protein expression, in vitro experiments were performed on cultured HL-1 cells and primary rat atrial myocytes. Our results showed that AGEs significantly decreased the expression of Cx43 and Cx40 in both HL-1 cells (Figure 3A-C) and primary rat atrial myocytes (Figure 3D-F), suggesting that AGEs have a negative effect on atrial gap junction proteins.

Role of AMPK Pathway in AGEs Regulating of Connexin Expression in Atrial Myocytes

As a key energy metabolism molecule in cell signaling, AMPK plays a critical role in the pathophysiological development of diseases. To explore the mechanism of AGEs regulating the expression of Cx43 and Cx40, we further determined the expression of p-AMPK and AMPK in the atrial tissue of two groups of rats. p-AMPK was significantly inhibited in diabetic atrial tissues while the total protein level of AMPK had no significant change (Figure 4A-C). In vitro studies show p-AMPK levels was significantly lower in the AGEs group compared to the BSA control group both in HL-1 cells and primary rat atrial myocytes, while the level of AMPK did not change significantly (Figure 4D-I), indicating AGEs is involved in AMPK inactivation.

(A) **HL-1 cells**

(B)

(C)

(D) **Primary rat atrial myocyte**

(E)

(F)

Figure 3 Altered protein expression levels of Cx43 and Cx40 in HL-1 cells and primary atrial myocytes treated with AGEs. (**A**) Representative blots of Cx43 and Cx40 proteins in each HL-1 cell group (n=6). (**B** and **C**) Densitometry analysis of Cx43 and Cx40 proteins in each HL-1 cell group (n=6). (**D**) Representative blots of Cx43 and Cx40 proteins in each primary rat atrial myocyte group (n=6). (**E** and **F**) Densitometry analysis of Cx43 and Cx40 proteins in each primary rat atrial myocyte group. *$p<0.05$.

## Effect of AICAR on AGEs-induced Dysregulation of Cx43 and Cx40

AGEs downregulate the expression of Cx43 and Cx40 in HL-1 and primary rat atrial myocytes, causing gap junction dysfunction and remodeling. Therefore, to investigate whether AGEs induce dysregulation of atrial junctional proteins by downregulating the expression of p-AMPK and thus the effect on this process, we analyzed the levels of Cx43 and Cx40 by AICAR treatment after 24 hours of AGEs incubation. AICAR could restore the expression of p-AMPK (Figure 5A-C). The results showed that AGEs significantly reduced the expression of Cx43 and Cx40 in HL-1 cells, whereas AICAR restored the expression of Cx43 and Cx40 (Figure 5A, D and E). These results suggest that AICAR prevents AGEs-induced Connexin dysregulation.

Knockdown of AMPK Decreased Expression of Cx43 and Cx40

To further investigate whether AMPK plays a regulatory role on connexin, we detected the protein levels of Cx43 and Cx40 after knocking down of AMPK α1 and α2 subunit respectively in HL-1 cells. The results showed that knockdown of AMPK α1 or α2 subunit significantly reduced the expression of Cx43 and Cx40 in HL-1 cells (Figure 6A-H).

AMPK Activation Increases Gap Junction Permeability

Altered gap junction channel proteins in cardiomyocytes are associated with the development of diabetic atrial fibrillation. Since activation of AMPK by AICAR upregulated the expression of Cx43 and Cx40, we further explored whether AICAR affects intercellular conduction in cardiac myocytes. We performed fluorescein yellow dye diffusion assays to detect gap junction function. AGEs treatment resulted in slower intercellular conduction as shown by a reduction in dye
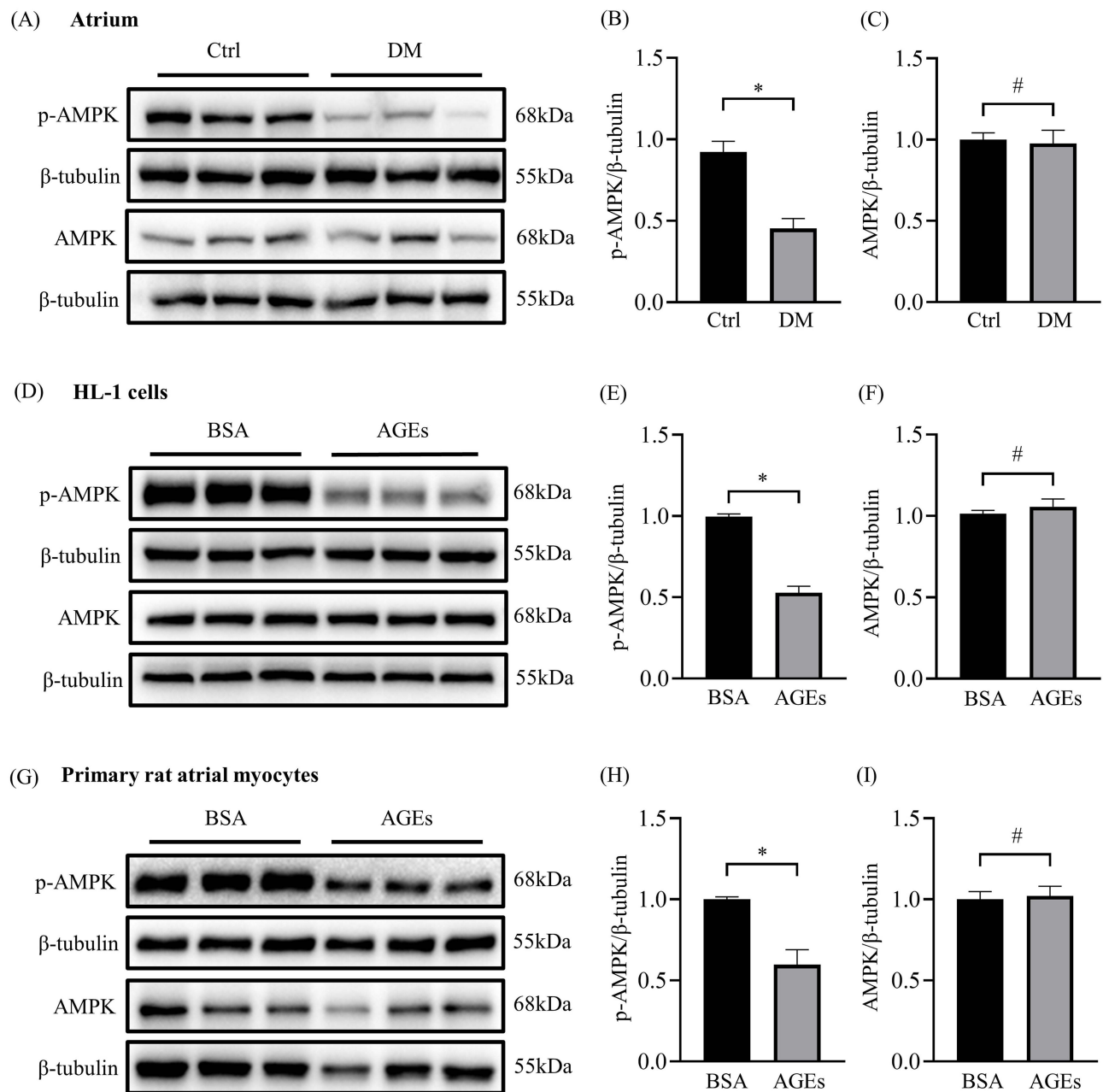
(A) **Atrium**

Figure 4 Inactivation of AMPK in atrial of diabetic rats and AGEs treated atrial cells. (**A**) Representative blots of p-AMPK (n=6) and AMPK proteins (n=12) in atrium of diabetic rat. (**B** and **C**) Densitometry analysis of p-AMPK (n=6) and AMPK (n=12) proteins in atrium of diabetic rat. (**D**) Representative blots of p-AMPK and AMPK proteins in each HL-1 cell group (n=6). (**E** and **F**) Densitometry analysis of p-AMPK and AMPK proteins in each HL-1 cell group (n=6). (**G**) Representative blots of p-AMPK and AMPK proteins in each primary rat atrial myocyte group (n=6). (**H** and **I**) Densitometry analysis of p-AMPK and AMPK proteins in each primary rat atrial myocyte group (n=6). *$p<0.05$, #$p>0.05$.

diffusion distance and AICAR significantly reversed this change (Figure 7). The result suggests that AMPK activation increases gap junction permeability in cardiomyocytes.

Discussion

The increased AGEs are closely associated with the development of diabetic-related AF.[12,21] However, the exact mechanism remains unknown. Our work focused on the study of the regulatory relationship between AGEs and gap junction proteins. The main findings of our study are: (1) Expression of AGEs and RAGE are increased in atrium of diabetic rats. (2) AGEs cause a decrease in the expression of Cx43 and Cx40 as well as abnormal distribution, which

(A)

(B) (C) (D) (E)
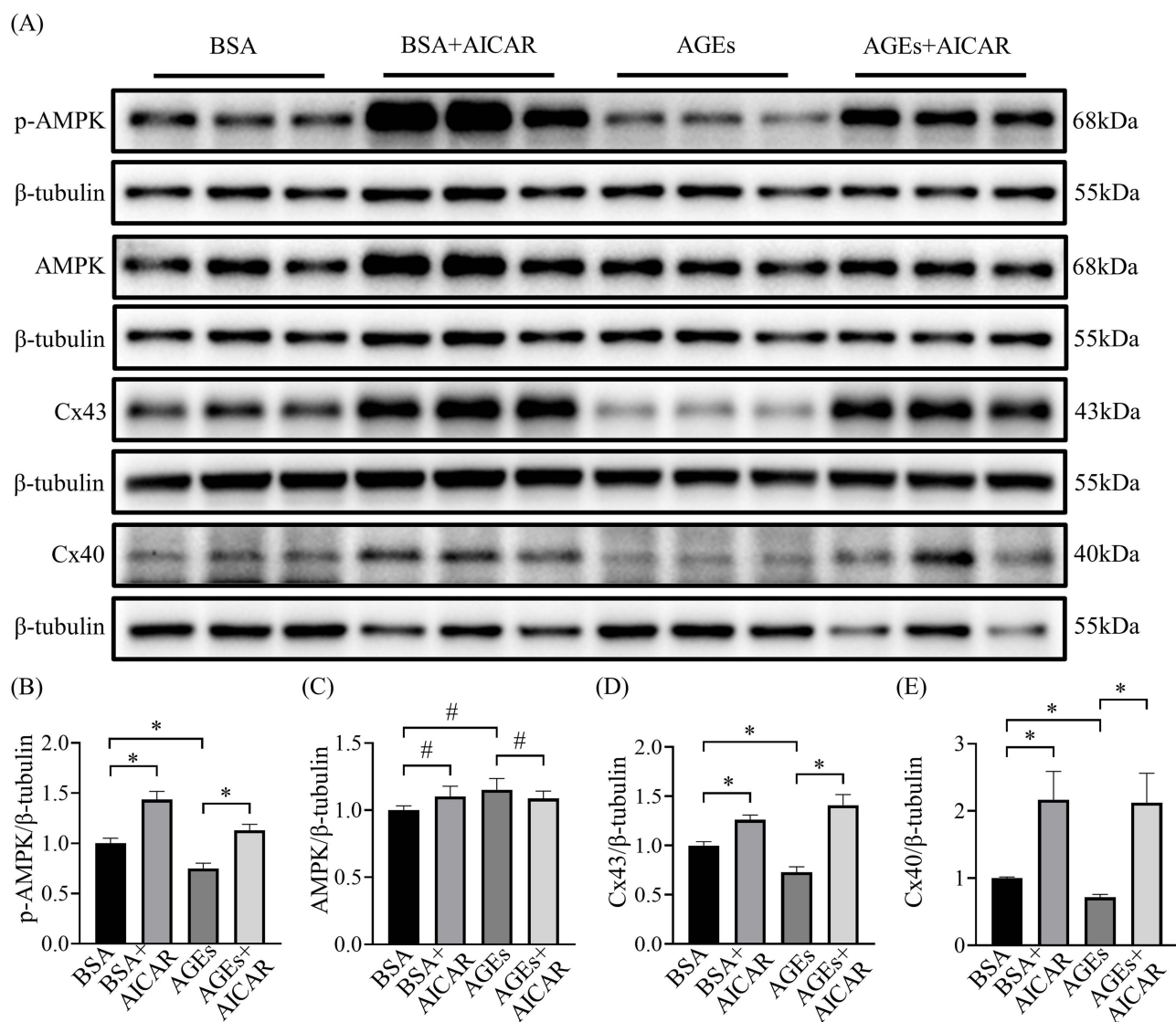
Figure 5 Effects of AMPK activation on AGEs-induced Cx43 and Cx40 alteration in atrial cells. (**A**) Representative blots of p-AMPK, AMPK, Cx43 and Cx40 proteins in each cell group (n=6-9). (**B-E**) Densitometry analysis of p-AMPK, AMPK, Cx43 and Cx40 proteins in each cell group (n=6-9). *$p<0.05$, #$p>0.05$.

subsequently leads to conduction dysfunction in atrial myocytes. (3) Knockdown of AMPK in vitro downregulates the expression of Cx43 and Cx40. (4) Phosphorylated AMPK was reduced by AGEs stimulation and activation of AMPK restored the expression of connexin and gap junction function. DM, a serious long-term metabolic disorder, promotes an excessive generation of AGEs, which is caused by the acceleration of glycation reaction in chronic hyperglycemia condition.[22] Consistent with previous studies, AGEs are substantially increased in combination with RAGE in DM and are involved in the pathogenesis of diabetic cardiovascular diseases.[12,23] Our results showed that the accumulation of AGEs in the atrial tissues of diabetic rats was significantly higher than the control group rats, along with a significant increase in RAGE. Several studies have proved that increased concentrations of AGEs in the circulation are closely linked with inflammation, microvascular dysfunction, and diabetic adverse cardiac remodeling.[24,25] Besides, it was revealed that AGEs could increase lipotoxicity by inhibiting sterol regulating element-binding protein (SREBP) signaling which played an important role in keeping balance between lipid and glucose, thus aggravating myocardial injury in diabetic cardiomyopathy.[26] Furthermore, a recent study demonstrated that AGEs treatment could prolong the action potential duration, decrease current density of $I_{Ca,L}$, I_{Kur} in HL-1 cells and reduce protein expression of $Ca_v1.2$, $K_v1.5$
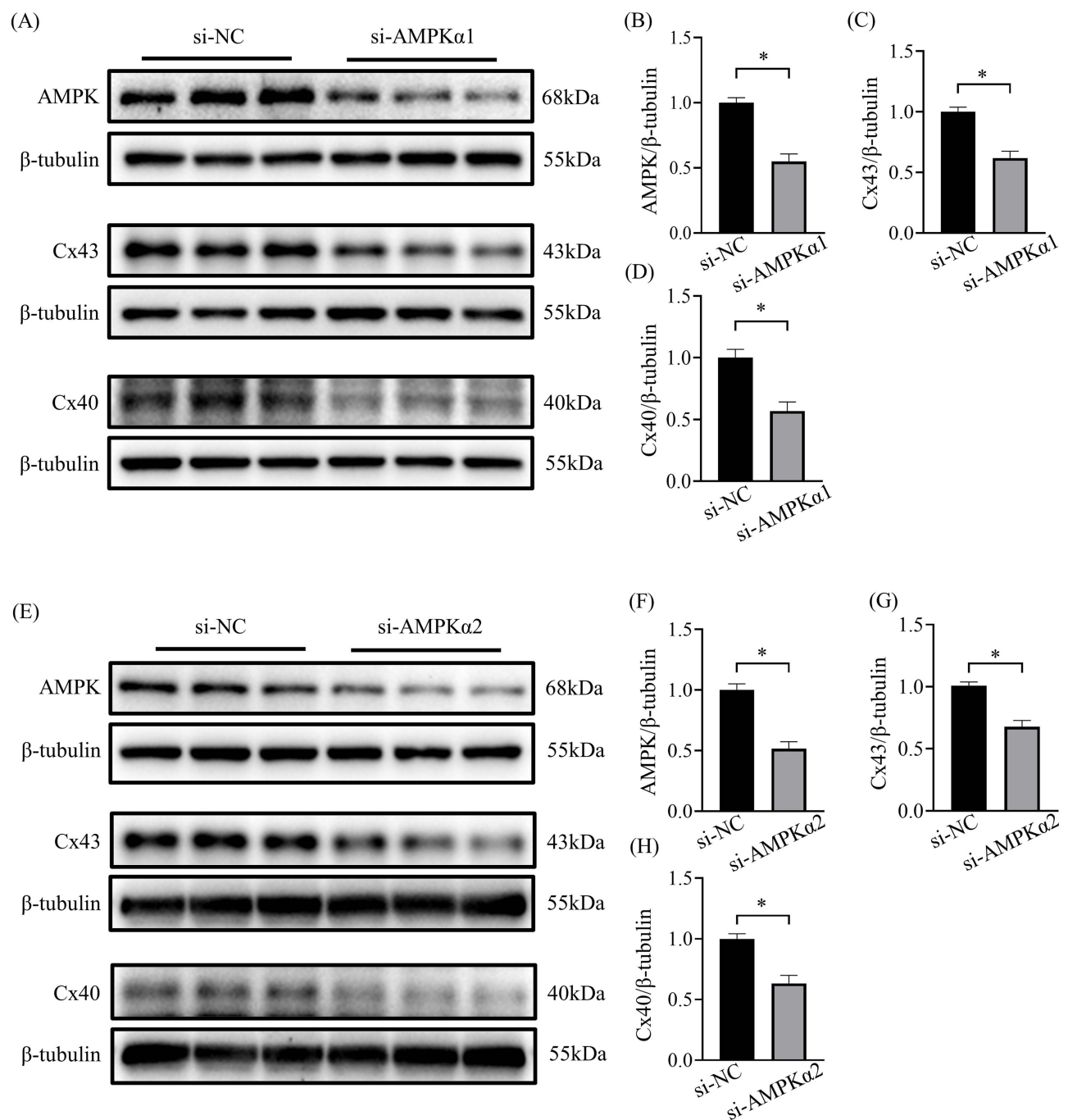
Figure 6 Decreased expression of Cx43 and Cx40 by downregulation of AMPK. (**A**) Representative blots of AMPK, Cx43 and Cx40 proteins in HL-1 cells transfected with AMPK α1 siRNA (n=6-9). (**B-D**) Representative densitometry analysis of AMPK, Cx43 and Cx40 proteins in HL-1 cells transfected with AMPK α1 siRNA (n=6-9). (**E**) Representative blots of AMPK, Cx43 and Cx40 proteins in HL-1 cells transfected with AMPK α2 siRNA (n=6-9). (**F-H**) Representative densitometry analysis of AMPK, Cx43 and Cx40 proteins in HL-1 cells transfected with AMPK α2 siRNA (n=6-9). *$p<0.05$.

both in atrium of diabetic mice and HL-1 cells, consequently increasing susceptibility to AF in diabetic mice.[12] Nonetheless, the concrete mechanism by which AGEs promote AF susceptibility remains to be explored.

Gap junctions (GJ) composed of connexins are essential for electrical coupling and rapid action potential conduction synchronously between cardiomyocytes. Growing evidence has shown that connexin remodeling induce altered atrial conduction heterogeneity in DM and reduced GJ function may play a critical role in promoting AF.[27–29] In patients with DM, the expressions of Cx43 and Cx40 were downregulated in the myocardium.[30] Yan.et al[31] discovered that long-term
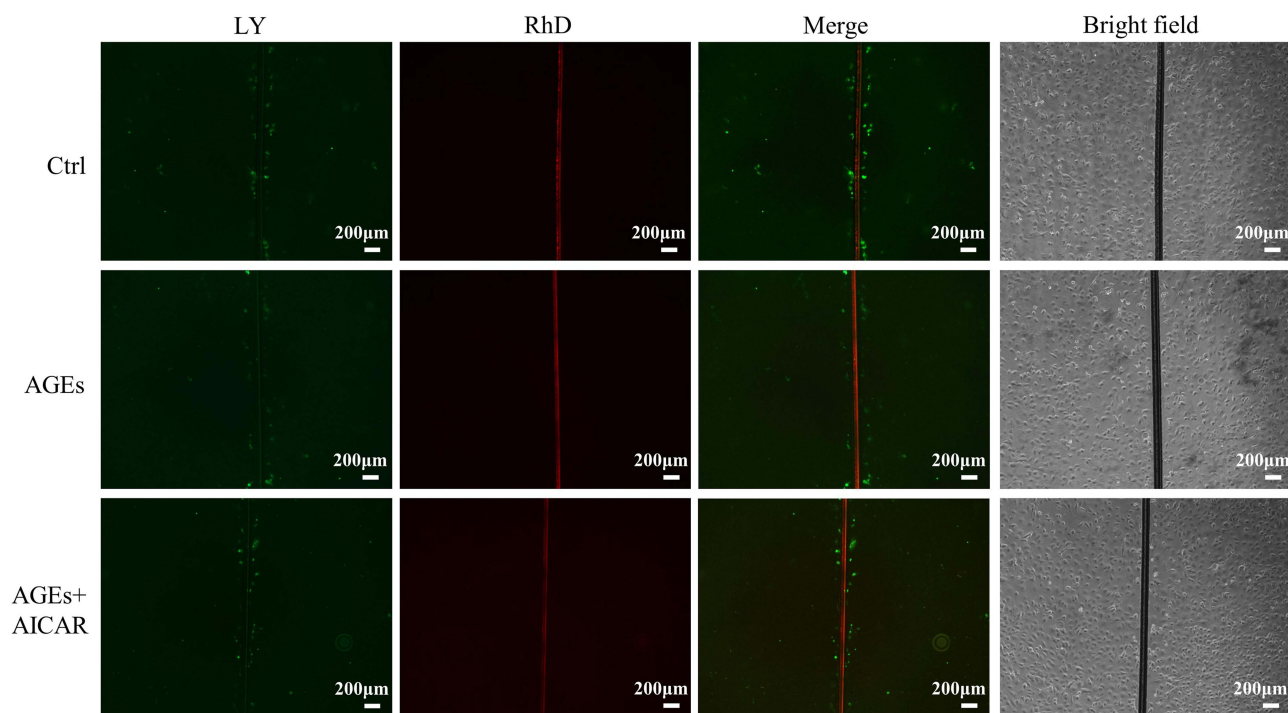
Figure 7 AMPK activation diminished AGEs-induced gap junctional dysfunction in primary atrial myocytes. Representative images of fluorescein yellow (LY) and rhodamine (RD) diffusion in each group of cells indicate lower diffusion through gap junctions in the AGEs group and increased diffusion in the AICAR group compared to the AGEs group.

JNK activation downregulates the protein level of Cx43 via c-jun suppressed transcriptional activity of the Cx43 gene promoter. Also, some other transcription factors were reported to induce AF through regulating the expression of atrial connexins.[29,32] However, the modulation of atrial gap junction in atrial though AGEs has not been reported. Our results revealed that Cx43 and Cx40 were significantly downregulated in diabetic rats and an increase in AGEs could cause a decrease in expression and function of Cx43 and Cx40 in atrial myocytes.

It has been demonstrated that the differences of protein expression between the left atrial appendage tissues of patients with AF and healthy individuals were closely related to energy metabolism.[33] AMPK is known as an "energy detector" in cells and closely associated with maintaining the balance of cellular energy supply and demand by influencing multiple aspects of cellular substance metabolism. A recent study proved that the deletion of AMPK in the atria promotes both the triggers and the substrate that predispose to the development of atrial fibrillation.[15] The p-AMPK, as active form of AMPK, plays a major role in vivo. The expression of p-AMPK was significantly decreased in diabetic mice and in cardiomyocytes cultured with high glucose.[34] Our work also proved that the expression of p-AMPK decreased in diabetic atrial tissue and AGEs cultured cells.

Dufeys's study explored that Cx43 in myofibroblast was significantly reduced in a mouse model of conditionally knocked out for myofibroblast-restricted AMPK α1.[13] Similarly, we knocked down AMPK in HL-1 cells and the expression of Cx40 and Cx43 was significantly downregulated. Hence, we hypothesize that AGEs impaired AMPK activity, resulting in a decrease in Cx43 and Cx40 and our experiments proved that both Cx43 and Cx40 were significantly decreased after intervention with AGEs in HL-1 cells and primary atrial myocytes.

AICAR has been one of the most commonly used pharmacological modulators of AMPK activity.[35] The protein level of Cx43 and Cx40 which was inhibited by AGEs was both restored by AICAR treatment for 24 hours after AGEs incubation for 24 hours and functional assays showed that AICAR also partially restored gap channel function. Different from post-AGEs treatment, AICAR pretreatment for 6 hours did not affect the Cx40 expression. This suggests different activation time or duration may affect the role of AMPK in AGEs induced – Cx40 regulation (data is shown in Supplement Figure 1). It has been reported that AMPK deficiency in mice atria promotes triggers and substrates that

induce AF by disrupting the expression of Gja5 (the gene encoding Cx40).[15] Consistently, we found Cx40 and Cx43 were decreased by AMPK downregulation with siRNA. However, the exact mechanism remains to be further explored.

There are several limitations in this study. First, the in vivo experiments suggest that AGEs reduce the expression of connexins, but no in vivo experiments were performed to show the role of AGEs in connexins and electrical conduction regulation. It would be best to test the conduction in vivo models by inhibiting AGEs formation or in ex vivo models of whole hearts or dissected atria. Second, immunofluorescence of Cx40 and Cx43 was not performed. Immunofluorescence can visually observe the expression and distribution of connexin. Third, the mechanisms underlying the inhibitory effects of AGEs on AMPK phosphorylation in atrium need to be further explored.

Conclusion

The present study demonstrates that AGEs decreased myocardial Cx43 and Cx40 expression through inhibition of the AMPK signaling pathway, which increase the susceptibility to DM-related atrial fibrillation. Reducing the production of AGEs and improving diabetic myocardial energy metabolism may influence AF by modulating gap junction function. Therefore, AGEs may be an intervention target for the prevention and treatment of diabetic atrial fibrillation.

Disclosure

The authors report no conflicts of interest in this work.

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