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Molecules and Cells

Inhibiting N-Cadherin-Mediated Adhesion Affects Gap Junction Communication in Isolated Rat Hearts

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Cadherin-mediated adherens junctions is impaired concomitant with a decrease in connexin 43 (Cx43) in diseases or pathological processes. We have investigated the acute effects of adherens junction impairment in isolated rat hearts by introducing Ala-His-Ala-Val-Asp-NH_2 (AHAVD, a synthetic peptide) as a specific inhibitor of N-cadherin. Effect of AHAVD on N-cadherin mediated adhension was analyzed by Cardiomy-ocyte aggregation assay. Laser confocal microscopy showed disrupted cell-cell contacts in cultured neonatal cardiomyocytes co-incubated with 0.2 mM AHAVD. In isolated adult rat hearts, Cx43 was redistributed along the bilateral of cardiomyocytes from the intercalated discs and significant dephosphorylation of Cx43 on serine368 occurred concomitantly with decreased gap junction (GJ) function in dose dependent manner after 1 h perfusion with AHAVD. These results indicate that impairing cad-herin-mediated adhesion by AHAVD rapidly results in Cx43 redistribution and dephosphorylation of serine368, thereby impairing GJ communication function.

INTRODUCTION

Adherens junctions, gap junctions (GJs) and desmosomes must be properly organized within the intercalated disc to mediate normal mechanical and electrical coupling between the individual cardiomyocytes in the heart (Li et al., 2006). N-cadherin, one of the most important transmembrane components, is a component of adherens junctions through its extracellular amino terminal region, forming homophilic adhesions between neighboring cells. N-cadherin-mediated adherens junctions stabilize the plasma membranes of adjacent cardiomyocytes, creating an environment favorable to the formation and maintenance of large arrays of GJs (Li et al., 2006). Recent studies confirm that severe conduction defects and increased susceptibility to ventricular tachyarrhythmias (VTs) are associated with GJ remodeling resulting from cardiac-restricted deletion of N-cadherin (Li et al., 2005; 2008). Adhesion is also disturbed under a number of pathological conditions, including healed infarctions (van den Borne et al., 2008) and arrhythmogenic right ventricular cardiomyopathy (ARVC) (Basso et al., 2006). Although each condition can be associated with a high risk of lethal ventricular arrhythmias, the initial effects of the adherens dysfunction on the functions of GJs remain to be elucidated.

GJs are composed of highly homologous proteins called connexins that facilitate direct communication and form low-resistance channels between adjacent cells by allowing passage of ions and small metabolites (Harris, 2007; Sohl and Willecke, 2004). GJs thereby in general act as pathways for direct cell-to-cell signaling and co-ordination of cellular activities in tissue. When gap junctional communication is compromised, normal tissue cannot function properly and disease can ensue (Severs et al., 2004). Connexin 43 (Cx43) is the most prominent protein subunit of the connexins in the ventricular myocardium of all mammalian species. The expression, distribution and phosphorylation of Cx43 are factors that affect the function of GJs (Poelzing and Rosenbaum, 2004; van Rijen et al., 2006). In cardiomyocytes, accumulation of Cx43 on the cell membrane and their formatting depend on the formation of adherens junctions (Hertig et al., 1996; Segretain and Falk, 2004). With regard to the important role of N-cadherin in GJs, we hypothesised that impairing N-cadherin-mediated adhesion can impair GJ remodeling under certain pathological conditions.

The cell adhesion function of cadherins is attributable to specific highly conserved amino acid sequences. One of these, the tripeptide HAV, is located in the first domain of N-cadherin, being the essential component of a cadherin recognition sequence (Blaschuk et al., 1990; Nose et al., 1990). A family of linear peptides harboring this motif inhibits a variety of cadherin-dependent processes (Burden-Gulley et al., 2009; Doherty et al., 1991; Williams et al., 2000). The amino acids that flank the HAV motif determine both the activity and specificity of the peptides, with those that mimick the natural HAVD sequence of N-cadherin being more effective inhibitors of N-cadherin function (Skaper et al., 2004; Williams et al., 2000).

We introduced AHAVD (the synthetic peptide sequence Ala-His-Ala-Val-Asp-NH_2) to inhibit in a highly selective manner N-cadherin-mediated adhesion function (Mysore et al., 2007; Skaper et al., 2004; Tang et al., 1998). Acute effects of impaired cell-cell adhesion has acute effects on both cultured neonatal rat cardiac cells and isolated adult rat hearts. AHAVD

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impaired N-cadherin-mediated cell-cell adhesion, resulting in the redistribution of Cx43 and dephosphorylation of Cx43 on serine368 (S368), decreases the permeability of gap junctions.

MATERIALS AND METHODS

Animals

Neonatal (1-2 days) and adult (16-20 weeks) male Sprague-Dawley-Javanovas rats were provided by the Animal Research Centre of Nanjing Medical University (China). The adult rats were housed at constant temperature (23 ± 2°C) and humidity (70 ± 5%) in a 12-h light/12-h dark cycle. They were given a standard diet for >2 days acclimation. Experiments were performed in accordance with the "Guide for the Care and Use of Laboratory Animals" (NIH Publication No. 85-23, National Academy Press, USA, revised 1996). The experimental protocol was approved by the Animal Care and Use Committee of Nanjing Medical University.

Antibodies and reagents

Primary antibodies study included the following: mouse monoclonal anti-Cx43 antibody (ab79010, Abcam, UK), rabbit polyclonal anti-N-cadherin antibody (sc-7939, Santa Cruz Biotechnology, USA) and rabbit polyclonal anti-phosphorylated Cx43 antibody (ab47368, Abcam, UK) specifically for detecting phosphorylated Cx43 at serine368 (pS368). Antibodies used in immunoblotting included all the aforementioned plus a mouse monoclonal anti-GADPH antibody (sc-51906, Santa Cruz, USA). Rhodamine-conjugated goat anti-mouse and FITC-conjugated goat anti-rabbit secondary antibodies were purchased from Sigma. HRP-conjugated goat anti-mouse and anti-rabbit secondary antibodies were purchased from Santa Cruz.

Reagents used were: penicillin/streptomycin (Amimed, Switzerland); 1-β-D arabinofuranosylcytosine, rhodamine-conjugated dextran (RD), and lucifer yellow (LY), purchased from Sigma and fetal calf serum from Hyclone. The synthetic peptide sequences were purchased from ChinaPeptides Company (China).

Cell culture and polypeptide handling

Cardiomyocytes were isolated from 1-2 day SD rats using enzymatic dissociation and purified by differential adhesion (Matsuda et al., 2005). The culture medium consisted of M199, 20% fetal calf serum and 1% penicillin/streptomycin. To inhibit fibroblast growth, 10 μM 1-β-D arabinofuranosylcytosine was added throughout the culture period. Cells were seeded in the dishes at 0.8-1 × 10^5 per cm^2. After 72 h culture at 37°C in a humidified 5% CO_2 in air incubator, the cells were replaced in HANKS medium containing 0.2 mM AHAVD (Mysore et al., 2007; Tang et al., 1998) for a further 15, 30 or 60 min before being fixed in 4% paraformaldehyde. After incubation with antibodies and fluorescent-labeled secondary antibodies, the cells were imaged in a confocal immunofluorescence microscopy (Zeiss LSM510 META, Germany). Control cells were incubated in low Ca^{2+} HANKS medium for 1 h. A nonsense peptide, Ile-Pro-Pro-Ile-Asn-Leu-NH_2 (IPPINL) (Chuah et al., 1991), serving as the negative control was processed in the same way at the same concentration.

Toxicity of both AHAVD- and IPPINL-handling was detected by the trypan blue staining test and compared with the non-peptide control. Neither AHAVD nor IPPINL was significantly toxic.

Cardiomyocyte aggregation assay

Effect of AHAVD on N-cadherin mediated adhension was analyzed reference to the method described previously (Mysore et al., 2007). In brief, the dissociated cardiomyocytes were counted, and approximately 500 μl of cell suspension (0.8-1 × 10^6 cells/ml) was used for each of treatments in HBS containing AHAVD or IPPINL in the dilutions of 0, 0.002, 0.02, 0.2 and 2 mM respectively. After 10-min incubation at 37°C, cells were rinsed with and suspended in culture medium and placed in a 37°C shaker at 500 rpm. Cell suspensions were plated onto slides 180 min later and observed under an invert microscope using an objective of 10×. The extent of aggregation was quantified as N0/Nt, where N0 was the number of cells not in aggregates in HBS containing 0 mM peptides, Nt was the number of cells not in aggregates at each test concentration.

Isolation and perfusion of rat hearts

Hearts from rats anesthetized with pentobarbital (70 mg/kg) were rapidly excised and placed in oxygenated Krebs-Henseleit buffer (K-H buffer) at 37°C in a Langendorff perfusion device (Lerner et al., 2000). They were perfused with oxygenated buffer at 37° at a constant rate of 7 ml/min by retrograde aortic flow. Simultaneously they were also submerged in a bath containing oxygenated buffer at 37°C to maintain a constant temperature. Ventricular epicardial electrograms were monitored at a continuous rhythm during the process. Any heart that demonstrated contractile dysfunction or any other evidence of injury was discarded in an initial 20 min equilibration period. Hearts were randomly assigned to 3 groups of after the 20 min stabilization interval. One group of AHAVD-perfused hearts (n = 12) was perfused with 0.2 mM AHAVD/perfusion buffer, the second group of IPPINL-perfused hearts (n = 13) with 0.2 mM IPPINL/perfusion buffer, and the control hearts (n = 12) with only the oxygenated buffer. To assess the dose effect of AHAVD, 24 more hearts were perfused with it at 0.002, 0.02 and 2 mM (n = 8, respectively). Snap-frozen sections from 4 hearts in each group were immunolabeled with primary and secondary Cx43 and N-cadherin antibodies (Saffitz et al., 2000). Specific immunofluorescence signals from N-cadherin, Cx43 and pS368 in the intercalated discs were imageded by confocal immunofluorescence microscopy. Heart tissues from each group were also collected for immunoblotting.

Loading of lucifer yellow and rhodamine dextran into whole hearts

Permeability of GJs in myocardial tissue was assessed in 4 of the hearts from those perfused with AHAVD in each concentration or with 0.2 mM IPPINL and 4 of the control hearts, using the method of Ruiz-Meana et al. (2001). After 60 min perfusion, a deep incision was made in the left ventricular wall with a surgical blade along from base to apex on the long axis. The whole hearts were placed in phosphate buffered saline (PBS) containing 2 fluorescent dyes, 2.5 mg/ml RD and 2.5 mg/ml LY at 37°C, with continuous gassing in O_2 for 10 min. After dye loading, the hearts were washed with PBS and rapidly frozen to -20°C. A laser confocal immunofluorescence microscope measured 60 μm transverse sections, and 2 separated 1024 × 1024 digital images per slice were obtained at 10×. The areas of distribution of LY and RD flourescence were quantified using Image-Pro Plus 6.0 software and expressed as fractions of the area of RD distribution to provide an index of GJ permeability.

Immunoblotting

The total contents of N-cadherin, Cx43 and pS368 were detected by immunoblotting as before (Johnson et al., 2002; Nakao et al., 2008). In brief, the lysates of cultured neonatal cardiomyocytes or the pulverized heart tissue from each group

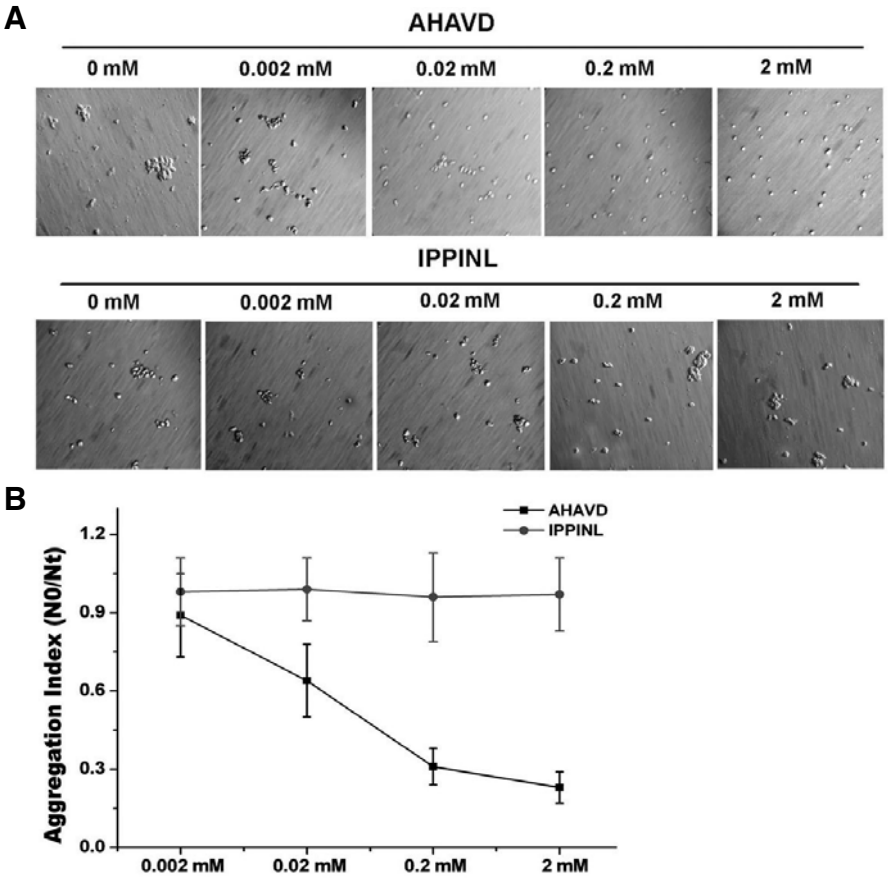

Fig. 1. Dose-dependent effects of AHAVD on N-cadherin-mediated aggregation of cardiomyocytes. (A) Representative images of isolated cardiomyocytes aggregate in culture medium after 10-minute handling in HANKS containing AHAVD or IPPINL in the dilutions of 0, 0.002, 0.02, 0.2 or 2 mM (n = 3). (B) Quantitative analysis of the extent of aggregation. N0 was the number of cells not in aggregates in HBS containing 0 mM peptides; Nt was the number of cells not in aggregates at each test concentration. Data are from three independent experiments.

were spun down, and proteins eluted by boiling for 10 min in SDS sample buffer (0.25 M Tris-HCl, pH 6.8, 4% SDS, 40% glycerol, and 0.002% bromophenol blue). To detect total Cx43, dephosphorylation involved incubating tissue lysates with calf intestinal alkaline phosphatase (10 U) at 37°C for 20 h (Goubaeva et al., 2007). Aliquots of protein were resolved by 10% SDS polyacrylamide gel electrophoresis and transferred semi-dry to nitrocellulose membranes. The membranes were blocked in 5% BSA in Tris-buffered saline-Tween 20 at room temperature for 2 h, and incubated with rabbit anti-Cx43 (1:4000), rabbit anti-pS368 (1:2000), mouse anti-GADPH (1:4000), and rabbit anti-N-cadherin anti-bodies overnight at 4°C. HRP-conjugated goat anti-mouse and anti-rabbit were used as secondary antibodies. GAPDH was used as the marker of protein loading. The densities of individ-ual bands were quantified as the ratio to GAPDH.

Statistical analyses

Data are expressed as mean ± SD. Differences between groups were analyzed using ANOVA, and considered significant at $p < 0.05$.

RESULTS

AHAVD peptide treatment disrupts cell-to-cell adhesion and results in Cx43 redistribution *in vitro*

The dose dependent effect of AHAVD on inhibiting the adherence of cardiomyocytes by examined the extent of cell aggregation (Fig. 1). The dissociated cardiomyocytes were suspended in the culture medium for 180 min after incubated with AHAVD or IPPINL in different dilutions for 10 min. Cells not in aggregates were counted under an invert microscope. The result show that ten-minute AHAVD (but not IPPINL) treatment can disrupt N-cadherin mediated adhesion. The treatments of 0.2 mM and 2 mM AHAVD are more efficient in inhibiting the aggregation of cardiomyocytes.

The effect of AHAVD impairing N-cadherin-mediated cell adhesion on the distribution of Cx43 was investigated by incubating the cultured neonatal cardiomyocytes with AHAVD. The cardiomyocytes were incubated with 0.2 mM AHAVD, IPPINL or HANKS medium for 1 h, double-immunolabeled with anti-N-cadherin and anti-Cx43 antibody, then examined by laser confocal microscopy. The control cells had abundant N-cadherin signals in the cell-cell connections, along with high Cx43 signals, but little Cx43 signal was detected in the cytoplasm. In contrast, cells treated with 0.2 mM AHAVD had more Cx43 in the cytoplasm. Broken connections between cells and a diffuse distribution of Cx43 on the plasmalemma were seen after 1 h treatment (Fig. 2). No detachment of cell-cell connections or redistribution of Cx43 was observed in cells incubated with 0.2 mM IPPINL.

Western immunoblotting (n = 4) was used to detect total N-cadherin and Cx43 in cell lysates; Figure 3 shows a typical blot. Neither N-cadherin nor total Cx43 protein was significantly changed after 60 min treatment with 0.2 mM AHAVD or IPPINL compared to the control group (the relative quantities of N-cadherin to GAPDH were 0.40 ± 0.11, 0.42 ± 0.09 and 0.39 ± 0.08, respectively, $P > 0.05$; and the relative quantities of Cx43 were 0.67 ± 0.18, 0.62 ± 0.14 and 0.68 ± 0.16, respectively, $P > 0.05$). Thus AHAVD inhibited N-cadherin-mediated adhesion and rapidly disrupted adherence junctions, thereby influencing the distribution of Cx43 in cultured cardiomyocytes.

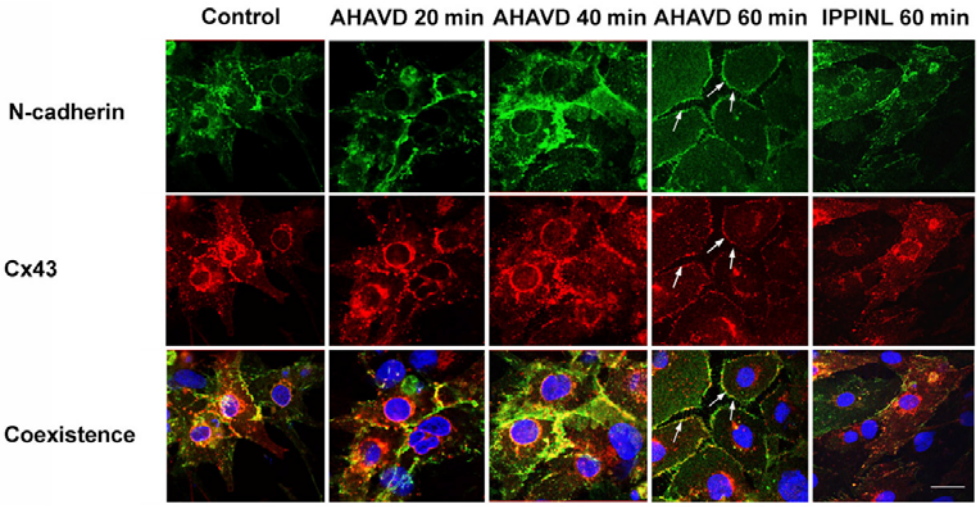

Fig. 2. Effects of AHAVD on N-cadherin-mediated adhesion and distribution of Cx43 in cardiac cells Normal cells and cells incubated with AHAVD and IPPINL were double-labeled with N-cadherin (green) and Cx43 (red). Nuclei were counterstained with Hoechst. Detached cell-cell adhesion and diffused distribution of Cx43 (arrows) are shown in cells incubated with AHAVD for 60 min. (bar = 20 μm for all the panels).

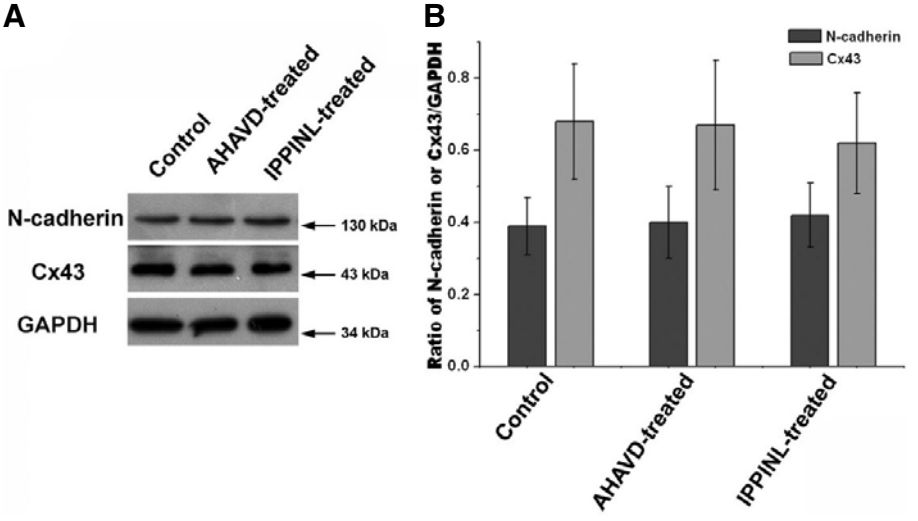

Fig. 3. Immunoblotting analysis of N-cadherin and total Cx43 in cells incubated with AHAVD or IPPINL (A). Representative immunoblots of N-cadherin and total Cx43 of lysates from AHAVD-treated, IPPINL-treated and control cells. GADPH was used as a loading control. (B). Densitometric scanning of N-cadherin and total Cx43 protein in the cells (n = 4, each group). Quantitative analysis showed no alteration of N-cadherin or total Cx43 in the cells incubated with AHAVD.

AHAVD inhibition of N-cadherin results in redistribution and dephosphorylation of Cx43 in isolated perfused rat hearts

As the results, the quantity and distribution of Cx43 and pS368 in the hearts perfused with AHAVD were examined. The efficiency of AHAVD was tested by perfusing isolated hearts with the oxygenated K-H buffer containing 0.002, 0.02, 0.2 and 2 mM AHAVD. Rapidly frozen tissue sections from 4 hearts of each group were double-labeled with N-cadherin and Cx43, and examined by laser confocal microscopy. Most of the N-cadherin signals were concentrated in the intercalated discs of the cells in tip-to-tip fashion in all the groups; only a few signals were distributed bilaterally in the cells. Cx43 co-localized with N-cadherin in the intercalated discs. However, apparent lateral distributions of Cx43 signals were seen in the tissues perfused with 0.2 or 2 mM AHAVD (Fig. 4A). Concomitant with the redistribution of Cx43, significant loss of pS368 immunofluorescence signal can be observed in the sections of heart tissue perfused with 0.2 or 2 mM AHAVD (Fig. 4B).

Total Cx43 and pS368 were measured by immunoblotting in 4 hearts from each group (Fig. 5). Compared with the control and IPPINL-perfused hearts, total Cx43 protein was not significantly changed in the AHAVD-perfused hearts at each concentration. However, the quantity of pS368 decreased when the concentration of AHAVD in the K-H buffer was increased, and a significant reduction of pS368 was seen in the hearts perfused with 0.2 or 2 mM AHAVD (0.26 ± 0.07 and 0.25 ± 0.07, respectively) compared with 0.2 mM IPPINL-perfused or control hearts (0.44 ± 0.05 and 0.41 ± 0.06, respectively, $p < 0.05$). Given the immunofluorescence and immonoblotting results, we would expect the GJ communication function to be impaired by inhibiting the N-cadherin-mediate adhesion with AHAVD.

AHAVD impairs GJ communication in isolated perfused rat hearts

The influence of redistribution of Cx43 and dephosphorylation of S368 caused by inhibiting N-cadherin-mediated adhesion with AHAVD on the GJ communication function was estimated. LY diffusion was assessed in the isolated rat hearts (n = 4) perfused with AHAVD in aforementioned dilutions, 0.2 mM IPPINL and K-H buffer only. Diffusion of LY to cells distant from those with disrupted sarcolemma in the tissue is shown in Fig. 6. The extent of LY and RD diffusion was analyzed in each slice. The difference in the area of diffusion between the 2 dyes was

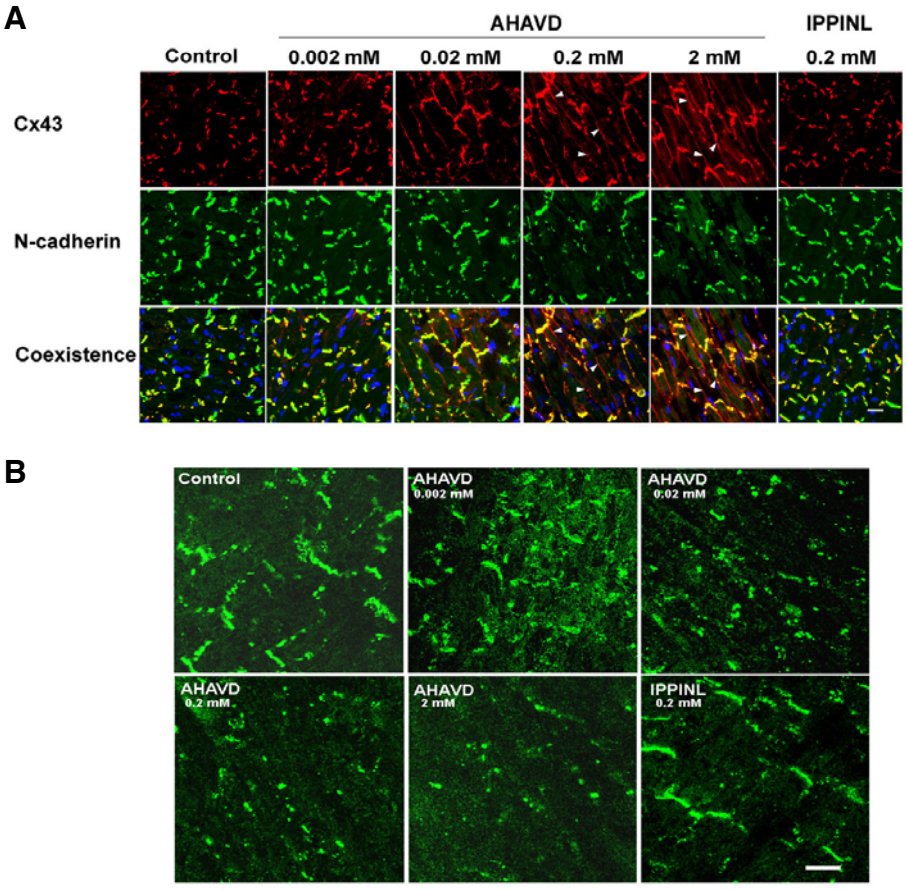

Fig. 4. AHAVD Affects Distribution of Cx43 and phosphorylation of Cx43 at S368 in Isolated Hearts. (A) Typical immunofluorescence images from the AHAVD- and IPPINL-perfused isolated rat hearts and oxygenated K-H buffer-perfused hearts. Double-labeling with N-cadherin (green) and Cx43 (red), showing dose related Cx43 redistribution to the bilateral edges of cardiac myocytes (arrow-heads) in the AHAVD-perfused heart tissue. Nuclei were counter-stained with Hoechst. (bar = 20 μm for all panels). (B) Representative immunofluorescence images from the hearts perfused with AHAVD at 0.002, 0.02, 0.2 or 2 mM, IPPINL (0.2 mM) and oxygen-ated K-H buffer, showing that AHAVD-perfusion reduces pS368 in a dose-dependent manner. (bar = 20 μm for all the panels).

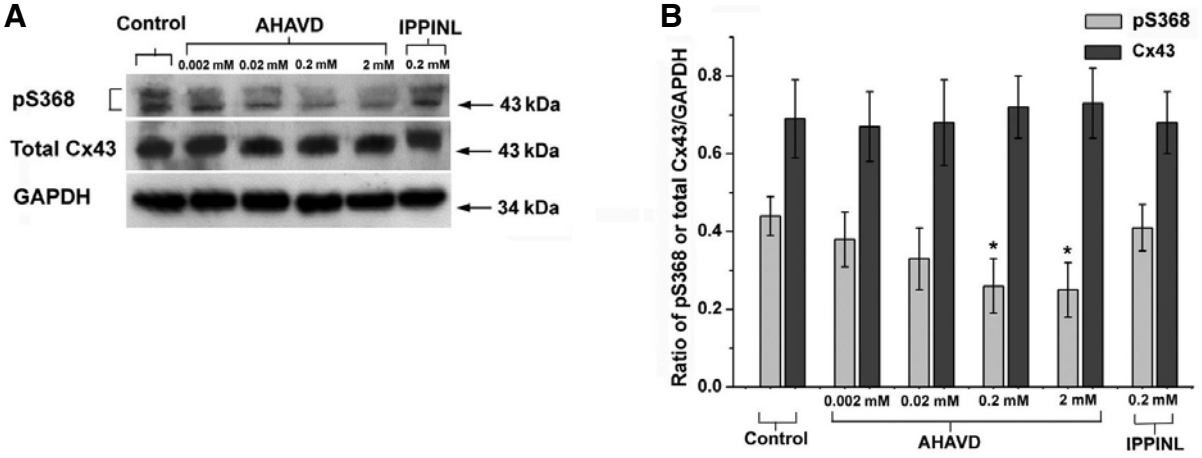

Fig. 5. Immunoblotting analysis of total Cx43 and pS368 in AHAVD-perfused hearts. (A) Representative immunoblot of total Cx43 and pS368 from hearts of control, AHAVD-perfused groups in the dilu-tions of 0.002, 0.02, 0.2 or 2 mM respectively, and 0.2 mM IPPINL-perfused group. GADPH was used as a loading control. (B) Densi-tometric scanning of total Cx43 and pS368 protein in the hearts (n = 4, each group). Quantitative analysis showed significant reduction of pS368 in hearts perfused with 0.2 or 2 mM AHAVD (* $P < 0.05$).

2.84 ± 0.78 in the IPPINL-perfused group, similar to that in the oxygenated buffer-perfused group (2.83 ± 0.65). However, when hearts were perfused for 60 min with AHAVD, the difference in the area of diffusion was decreased when the concentration of AHAVD increased in the K-H buffer and a significant discrimination can be observed in 0.2 mM or 2 mM AHAVD perfused hearts (1.37 ± 0.33, $P < 0.05$ and 1.1 ± 0.23, $P < 0.01$ respectively, compared with control groups). Therefore, inhibiting the N-cadherin-mediated adhesion by AHAVD rapidly impairs communication between cardiomyocytes through the GJs. The result hints at a significant lost of functional GJs in the intercalated discs, which is accordance with the redistribution and dephsophorylation of S368.

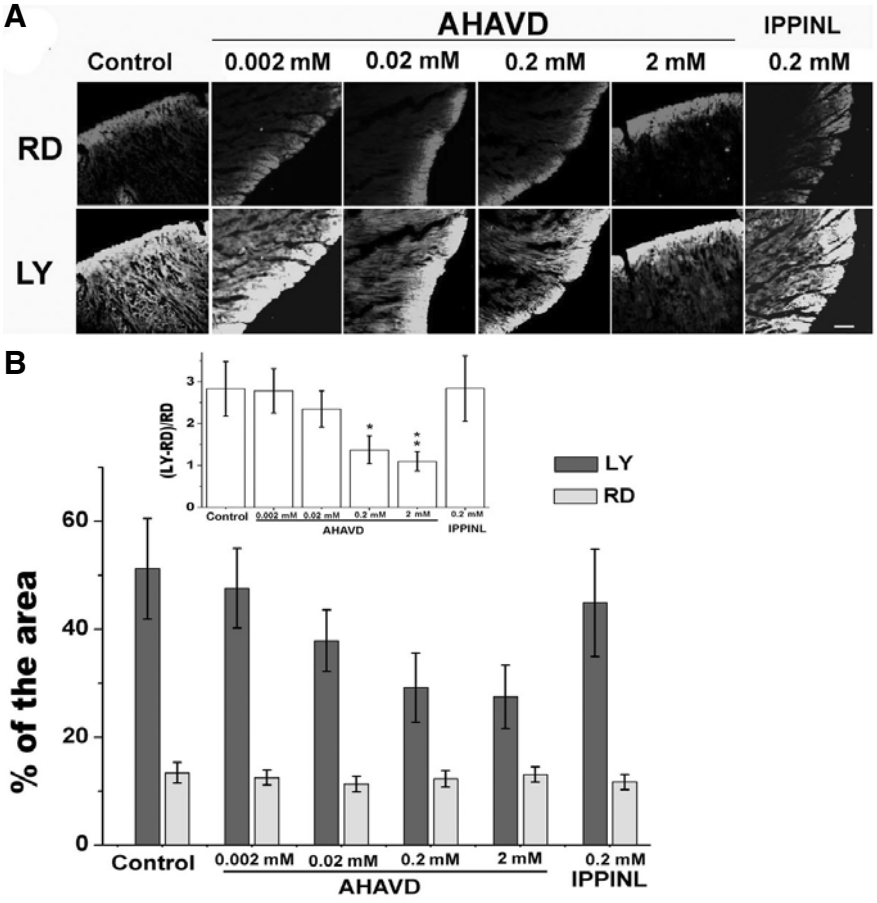

Fig. 6. AHAVD Affects LY Diffusion Through GJs in Intact Rat Hearts. (A) Cell-to-cell dye diffusion in AHAVD-, IPPINL-perfused and control rat hearts. Bar = 100 μm, for all the panels. Addition of rhodamine dextran (RD) corresponds to cells with disrupted sarcolemmas, which were mainly distributed along the borders of the myocardial incision exposed to the dyes. Infusion with LY represents intercellular dye diffusion through GJ to cells distant from the border of the incision. (B) Quantitative analysis showed the reduction of LY diffusion areas in the hearts perfused with AHAVD in different dilutions. Inset: relative difference between both these areas in the same groups (n = 4 for each group; *P < 0.05, **P < 0.01).

DISCUSSION

By interrupting the N-cadherin adhesion with AHAVD, the initial effects of impairing cell mechanical coupling on the distribution of Cx43, phosphorylation of Cx43 on serine368, and communication through the GJs between cardiomyocytes could be followed. A decreased permeability of GJs was observed in AHAVD-perfused isolated rat hearts. We also confirmed that impaired cell-to-cell adhesion between cardiomyocytes promptly results in removal of Cx43 from the intercalated discs and redistribution to the bilateral sarcolemma, accompanied by decreased phosphorylation of Cx43 on serine368. Interestingly, total Cx43 did not decrease when cell-to-cell adhesions were impaired with AHAVD.

Cell-cell adhesion between cardiomyocytes is mediated by N-cadherin. The classical cadherins contain an evolutionarily conserved His-Ala-Val (HAV) sequence. Linear peptides harboring the HAV motif inhibit a variety of cadherin-dependent processes (Burden-Gulley et al., 2009; Mysore et al., 2007). The amino acids flanking the HAV motif determine both the activities and specificities of these peptides, and the peptides that mimick the natural HAVD sequence of N-cadherin proved more effective inhibitors of N-cadherin function (Skaper et al., 2004; Williams et al., 2000). Inhibition of the adhesion function of N-cadherin by these peptides affects cadherin-mediated processes, such as neurite outgrowth (Burden-Gulley et al., 2009), embryo compaction (Blaschuk et al., 1990), cell aggregation (Willems et al., 1995), cell differentiation and survival (Chuah et al., 1991).

Impaired adherens junctions in both diseases and animal models are associated with remodeling of GJs and the high risk of ventricular arrhythmias (Ferreira-Cornwell et al., 2002; Kaplan et al., 2004; Li et al., 2008), but the agent linking abnormal adherens junctions to GJ remodeling was not identified. Mechanical coupling mediated by cadherins may be important in forming and maintaining GJs (Li et al., 2006; Noorman et al., 2009). Our results indicate that impairment of adherens junctions results in the prompt redistribution of Cx43, which may be implicated in the loss of GJs from intercalated discs.

The phosphorylation/dephosphorylation of Cx43 regulates the internalization, degradation and channel-gating properties of GJs (Laird, 2005; Moreno, 2005). In acute ischemia, dephosphorylation of Cx43 is important in the disorganization and electrical uncoupling of ventricular cells (Beardslee et al., 2000; Jain et al., 2003; Lampe and Lau, 2004; Turner et al., 2004). However, regulation of GJ communication function by phosphorylation of Cx43 remains to be elucidated. Jose et al. (2006) discovered that reducing (or eliminating) pS368 in cells by expressing the carboxyl terminus of Cx43 as a distinct protein, treatment with a PKC inhibitor, or mutation of S368 can signifycantly reduce the selective permeability of GJ to a small cationic dye. In analyzing the effect of inhibiting N-cadherin-mediated adhesion on pS368, pS368 decreased significantly in the intercalated discs of AHAVD-perfused hearts. The lucifer yellow transfer test confirmed the impairment of GJ communication accompanied by the redistribution of Cx43 and the loss of pS368 in the AHAVD-perfused rat hearts.

The relationship between GJ permeability and low-resistance

electrical communication through GJs is complex, and regulatory mechanisms can have opposing effects on electrical and dye coupling (Kwak and Jongsma, 1996; Kwak et al., 1995), possibly because of opposing effects on the probability that the GJ channels are in an open state and on the size of the opening. Despite controversy about the relationship between GJ communication and electrical channel function, the association between GJ electrical uncoupling and arrhythmias has been established (Gutstein et al., 2001; Lerner et al., 2000). Cx43 reduction results in slower conduction and enhancement of the development of stable re-entrant circuits, which is a major mechanism behind the occurrence of ventricular tachyarrhymias in related diseases (Lerner et al., 2000; Musil and Goodenough, 1990). We found impaired fascia adherence and consequent redistribution of Cx43 and reduction of pS368 may result in a reduction of functional GJs in intercalated discs. This result demonstrates the importance of N-cadherin-mediated adhesion in maintaining electrical coupling between cardiomyocytes. We believe that impaired adherence junctions increase the risk of ventricular tachyarrhymias due to more dysfunctional GJs in the intercalated discs.

CONCLUSION

The peptide AHAVD acutely interrupts N-cadherin-mediated adhesion, resulting in rapid redistribution and dephosphorylation of Cx43 on serine368, and decreasings the GJs communication in isolated rat hearts. These results demonstrate the importance of mechanical coupling between cardiomyocytes in sustaining the communication function of GJs. Further investigation of the arrhythmogenic effect of impaired adherens junctions on electrical coupling should throw new light on the mechanism of arrhythmias.

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