Short-Duration Therapeutic Hypothermia Causes Prompt Connexin43 Gap Junction Remodeling in Isolated Rabbit Hearts

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Background: Whether connexin43 gap junctions (Cx43 GJs) and spatial heterogeneity of conduction velocity (CV) restitutions are altered in hearts during moderate (MH; 33°C) and severe (SH; 30°C) hypothermia remains unclear.

Methods and Results: Using an optical mapping system, ventricular CV was evaluated by S1 pacing in 29 Langendorff-perfused isolated rabbit hearts at baseline (37°C, n=9), 30-min MH (n=6), 30-min SH (n=9), and rewarming (R, 30-min SH followed by 30-min 37°C, n=5). After CV evaluation, myocardium was collected to measure the level and distribution of non-phosphorylated (NP-Cx43) and total (T-Cx43) Cx43 by immunoblotting and immunofluorescence microscopy. In 6 additional hearts, Cx43 GJ remodeling was evaluated at 30-min SH with (n=3) or without (n=3) pretreatment of a protein kinase C (PKC) inhibitor. CV slowing and spatial heterogeneities of CV restitutions were enhanced in MH and SH hearts. NP-Cx43 was downregulated in MH (P=0.002) and SH (P<0.001) hearts. NP-Cx43 levels among 4 different ventricular sites became inhomogeneous in MH (P=0.017) and SH (P=0.046) hearts. However, T-Cx43 levels were unchanged. The percentages of lateralized NP- and T-Cx43 were increased in MH, SH, and R hearts. Pretreatment of PKC inhibitor attenuated SH-induced NP-Cx43 lateralization (P=0.0495).

Conclusions: Short-duration (30min) therapeutic hypothermia causes prompt Cx43 GJs remodeling, in which the PKC pathway is involved. Rewarming abolished hypothermia-induced conduction disturbance, while Cx43 GJs lateralization did not completely recover. (Circ J 2011; 75: 1706–1716)

Key Words: Conduction velocity; Connexin43; Hypothermia; Immunohistochemistry; Optical mapping

Therapeutic hypothermia (TH) has been shown to improve neurological recovery in patients resuscitated from cardiac arrest due to ventricular fibrillation (VF).1 Clinical guidelines accordingly recommend that unconscious adult patients with spontaneous circulation after out-of-hospital cardiac arrest should be cooled to 32–34°C for 12–24h.2 However, TH per se might potentiate the occurrence of ventricular tachycardia (VT) and VF, and the mechanisms remain unclear.3 In addition to slow conduction velocity (CV), heterogeneous and anisotropic propagation of excitation impulse might also cause unidirectional conduction block, leading to reentry ventricular tachyarrhythmia during TH.4,5 We have recently reported that severe hypothermia (SH; 30°C) enhanced the spatial heterogeneity of CV restitutions (CVRs), facilitated the onset of discordant alternans, and increased the vulnerability of pacing-induced VF in isolated rabbit hearts.6 These findings suggest that conduction disturbance (ie, slow and heterogeneous conduction) during TH might play a pivotal role in initiating VT/VF, which might offset the neurological benefits of TH.

One important determinant of cardiac CV is cell-to-cell coupling, which is mediated by gap junctions (GJs) constructed from connexin (Cx) proteins.7 In human, ventricular cardiomyocytes mainly express Cx43 whose half-life is between
Therapeutic Hypothermia and Gap Junction Remodeling

1 and 3 h. In subacute/chronic models of failing, infarcted, and hypercholesterolemic hearts, conduction disturbance is associated with GJ remodeling, including alteration in expression and distribution of Cx43 in ventricles. Yeh et al recently found that even within 20–40 min of cardiopulmonary bypass, including application of TH, Cx43 in human atrium was downregulated and redistributed. These reports raise the possibility that GJ coupling in ventricles might have altered shortly after the implementation of TH, and consequently contributed to the conduction disturbance during TH. Furthermore, the phosphorylation status of Cx43 could influence GJ function and CV. Whether the phosphorylation status of Cx43 changes during TH remains unclear.

In this study, using an optical mapping system, we investigated the ventricular conduction properties (CV and spatial heterogeneity of CVRs) at baseline (37°C), 30-min moderate hypothermia (MH: 33°C), 30-min SH (30°C), and rewarming in Langendorff-perfused isolated rabbit hearts. Expression and distribution of total (T-Cx43) and non-phosphorylated (NP-Cx43) Cx43 at the optical-mapped area were analyzed using immunoblotting and confocal microscopy. We hypothesized that short-duration TH promptly causes a temperature-dependent GJ remodeling that contributes to conduction disturbance. Furthermore, rewarming the hearts from SH can reverse the GJ remodeling and conduction disturbance induced by SH. Previous studies suggest that hypothermia might activate the protein kinase C (PKC) pathway, which can alter the assembly and trafficking of Cx43. We therefore evaluated whether a PKC inhibitor (PKCI) could attenuate TH-induced Cx43 GJ remodeling.

Methods

The research protocol was approved by the Institutional Animal Care and Use Committee of Taichung Veterans General Hospital.

Langendorff Preparation of Isolated Rabbit Hearts

New Zealand white rabbit hearts (n=41; M/F: 34/7; 2.5–4.4 kg) were excised under general anesthesia. The ascending aorta was cannulated and perfused with 37°C Tyrode’s solution composed of (in mmol/L): 125 NaCl, 4.5 KCl, 0.5 MgCl₂, 24 NaHCO₃, 1.8 NaH₂PO₄, 1.8 CaCl₂, 5.5 glucose, and albumin (40 mg/L), respectively. Coronary perfusion pressure and flow rate were 60–65 mmHg and 35–45 ml/min, respectively. The hearts were perfused and superfused in a thermostatically controlled tissue bath.

Optical Mappings

Using a 2-camera optical mapping system, epicardial acti-
Oxations in the anterior and posterior aspects of the hearts were simultaneously mapped. The hearts were stained with di-4-ANEPPS, and excited with 4 light-emitting diode modules. Induced fluorescence was collected by 2 image-intensified charge-coupled cameras (model C A D1-0128T). Optical signals were gathered at 3.85-ms sampling intervals, acquired from 128×128 sites simultaneously over a 30×30 mm² area in each aspect of the heart. For each optical recording, data were acquired continuously for 3.85 s. To minimize motion artifacts, cytochalasin-D (5 μmol/L), an excitation–contraction uncoupler, was used.

A pair of hook bipolar electrodes was inserted into the RV outflow tract for S₁ pacing. During S₁ pacing with a steady state (>50 beats after the onset of pacing), we evaluated CV in squares (10×10 mm) located at the centers of the anterior (A) and posterior (P) aspects of both ventricles (RV and LV), namely, at the RVA, RVP, LVA, and LVP (4 sites, Figures 1A, B). In each square, epicardial conduction perpendicular to the propagating wavefronts was selected to measure the CV (cm/s) using depolarization isochronal maps (Figure 1B). The mean of the CVs from these 4 sites became the CV of the heart. To induce MH (33°C), we switched the thermostatic system to the TH setting of 33°C. The superfusate was also quickly replaced with 33°C Tyrode’s solution. When the tissue bath temperature reached 33°C, an additional 5 min (stabilized at 33°C) was allowed to ensure the homogeneity of tissue temperature, and then the study protocol was started. The methods to induce SH (30°C) were the same. To rewarm (37°C) the heart, the procedures were reversed.

**Tissue Preparation**

Blocks of myocardium from the RVA, RVP, LVA, and LVP (corresponding to the squares for CV measurement) were excised and cryoembedded immediately after S₁ pacing protocol for immunoblotting and immunofluorescent microscopic study.

**Immunoblotting**

The snap-frozen myocardium was homogenized, and protein was extracted with an alkaline extraction method as previously described. The protein was loaded with an equal amount (20 μg) per lane for electrophoresis. The snap-frozen myocardium was homogenized, and protein was extracted with an alkaline extraction method as previously described. The protein was loaded with an equal amount (20 μg) per lane for electrophoresis.
After transferring the protein to nitrocellulose, membranes were blocked and probed for 60 min with mouse anti-T-Cx43 (1:250, BD Biosciences, Lexington, KY, USA) and mouse anti-NP-Cx43 (1:250, Cx43 dephosphorylated at Ser\(^{368}\); Zymed, California, CA, USA) antibodies.\(^{10,14}\) Membranes were washed and then incubated with a goat anti-mouse alkaline phosphatase-conjugated secondary antibody (1:5,000; Chemicon, Temecula, CA, USA). Anti-Na\(^+\)v antibody (1:100, Alomone Labs, Jerusalem, Israel) was used to evaluate the Na\(^+\) channel protein expression.\(^{21}\) The enzymatic activity was revealed with alkaline phosphatase chemiluminescent substrates (Amresco, Solon, OH, USA) and quantified by densitometry.\(^{10,14}\) Band densities were normalized to the density of GAPDH to correct the variations in protein loading, and were quantitatively expressed as arbitrary units (a.u.). In each heart, the protein levels were the means of the 4 sites.

**Immunohistochemistry Study and Confocal Microscopy**

Myocardial blocks were cryosectioned (12\,\mu m thick) and primarily immunostained with mouse anti-T-Cx43 and anti-NP-Cx43 monoclonal antibodies as mentioned above.\(^{10,14}\) Donkey anti-mouse immunoglobulin conjugated to CY3 (Chemicon) was used to visualize immunolabeled Cx43 by confocal microscopy (Leica TCS SP).\(^{14}\) Each image consisted of 1,024\times 1,024 pixels (250\times 250\,\mu m) using a \times 40 objective lens and zoom 1.55 computer setting. Projection views of consecutive optical sections were taken at 0.4-\mu m intervals through a thickness of 2.4\,\mu m within the myocardium. In each specimen, we randomly selected at least 4 images with myocardial fiber orientation parallel to the long axis of cells for analysis.\(^{10}\) In these areas, cell border and Cx43 GJs in the intercalated disk could be easily identified (Figures 3A, 5A, MH images as examples).\(^{10,12}\) Assisted by image analysis software (QWIN, Leica), we measured (1) the percentage of total tissue area occupied by Cx43 immunoreactive signals, and (2) the percentage of Cx43 signal located outside the end-to-end cell junctions (lateralization) (Figures 3A, 5A).\(^{10,12,22}\) In each heart, the percentages were means of the 4 sites.

**Subcellular Fractionation**

Subcellular fractionation was performed to evaluate the amount of T-, NP-Cx43, and Na\(^-\)1.5 proteins in the cytoplasm and membrane compartments. Myocardium was chopped, homogenized, and fractionated into cytosolic and membrane fractions with CNM compartment protein extraction kit (BioChain Institute, Hayward, CA, USA).\(^{23}\) These fractions were separated by SDS–10% PAGE, and proteins were detected by immunoblotting using antibodies as mentioned above. The membrane and cytosolic fractions were checked for specificity and relative loading by mouse anti-Na/K ATPase (Abcam, Cambridge, UK) and anti-GAPDH (Chemicon) antibodies, respectively.

**Study Protocols**

**Protocol I: Effects of TH on Ventricular Conduction and Cx43 GJ Remodeling**

**Protocol IA: Baseline (37°C) Ventricular Conduction and...**
Cx43 GJ (B Group, n=9)  S1 pacing (2× diastolic threshold) was used to determine the CV at PCLs of 400, 350, 300, 250, 200, 180, and 160 ms. Afterwards, ventricular tissue from the 4 sites were quickly frozen in liquid nitrogen and embedded in Tissue-Tek OCT compound (Sakura Finetek, Torrance, CA, USA) for immunoblotting, subcellular fractionation, and immunofluorescence microscopy.

Protocol IB: 30-Min MH (33°C) on Ventricular Conduction and Cx43 GJ Remodeling (MH Group, n=6) The hearts were cooled from baseline to MH. Once a steady temperature of 33°C had been achieved in the tissue bath, the hearts were kept at 33°C for a further 30 min. Subsequently, S1 pacing protocol was performed and then the ventricular tissues were collected for studies as described in Protocol IA.

Protocol IC: 30-Min SH (30°C) on Ventricular Conduction and Cx43 GJ Remodeling (SH Group, n=9) This protocol was the same as protocol IB, except SH was used instead of MH.

Protocol ID: Rewarming (30-Min 30°C Followed by 30-Min 37°C) on Ventricular Conduction and Cx43 GJ Remodeling (R Group, n=5) The hearts were sequentially cooled from baseline to a 30-min SH, followed by rewarming from SH to a 30-min rewarming at 37°C. Subsequently, S1 pacing protocol was performed and then the ventricular tissues were collected for studies as described in Protocol IA.

Protocol II: Effects of PKCI on SH-Induced Cx43 GJ Remodeling (n=6) This protocol was the same as protocol IC, except a PKCI (chelerythrine 5 µmol/L) was added to the perfusate 10 min before cooling (n=3). Control hearts (n=3) received vehicle only (250 µl DMSO). The ventricular tissues were collected for immunoblotting and immunofluorescence microscopy.

Protocol III: Effects of SH (30°C) on Subcellular Distribution of Nav1.5 Protein (n=6) Changes in Na+ channel expression and internalization might lead to conduction disturbance. Protocol III was performed to exclude the possibility that SH-induced conduction disturbance also resulted from structural remodeling in the Na+ channel. The hearts were subjected to baseline preparation (as per Protocol IA, n=3) or 30-min SH (as per Protocol IC, n=3). LV tissue was collected for subcellular fractionation.

Statistical Analysis
Data are presented as mean±SD. Non-parametric test and chi-square analysis with Yates correction were used to compare the data between and within groups. A probability value of ≤0.05 was considered significant.
Results

Effects of MH and SH on Ventricular CV and Spatial Heterogeneities of CVRs

Compared with B group hearts, the MH and SH hearts showed a significant decrease in CV at all PCLs, except during MH at PCL of 400 ms (Figures 1B, C). Furthermore, SH hearts revealed a slower CV than MH hearts (P<0.05 for all PCLs) (Figure 1C), as evidenced by more crowding of the lines in the isochronal maps of SH hearts (Figure 1B). The CVs in R group hearts were not different from those in B hearts (P=NS for all PCLs) (Figures 1B, C). "Spatial heterogeneity of CVRs" was not enhanced in B (22±13, 26±9, 16±5, and 22±9 cm/s, for RVA, RVP, LVA, and LVP, respectively, P=0.261) or R (22±8, 30±10, 24±11, 17±7 cm/s, for 4 sites, respectively, P=0.209) hearts (Figure 1D). However, this heterogeneity significantly increased in MH (10±2, 14±1, 9±2, 28±19 cm/s, for 4 sites, respectively, P=0.209) hearts (Figure 1D). During S1 pacing, no sustained VF was induced in B or R hearts. However, 4 sustained VF episodes were induced (PCLs: 200–250 ms) in 4 SH hearts (hearts 3, 5, 8, 9; P=0.023 compared to B group), while only one VF episode was induced (PCL: 200 ms) in a MH heart (heart 1; P=0.205 compared to B group).

Effects of MH and SH on T-Cx43 Expression and Distribution

T-Cx43 Expression

Compared with B hearts, MH (P=0.123), SH (P=0.711), and R hearts (P=0.841) showed similar T-Cx43 levels (Figures 2A, B). Differences in T-Cx43 between any 2 of the 4 groups were insignificant (Figure 2B). Although there was no significant change in total band intensity in the MH and SH hearts, we observed a decrease in band density at lower molecular mass (≈41 kDa, the P0 band in Figure 2A), suggesting a decrease in hypophosphorylated isoform of T-Cx43 in MH and SH hearts. T-Cx43 levels among the 4 sites were similar in B (P=0.585), MH (P=0.718), SH (P=0.788), and R (P=0.542) hearts (Figure 2C).

T-Cx43 Distribution

A total of 503 areas (B: 148, MH: 98, SH: 176, R: 81 images) from 4 ventricular sites were selected for morphometric analysis. The percentages of total tissue area occupied by T-Cx43 immunoreactive signals were similar in MH (5.8±0.5%, P=1.0), SH (5.6±0.7%, P=0.771), and R (5.6±0.5%, P=0.770) hearts compared to B (5.7±1.1%) hearts (Figures 3A, B), which was consistent with the immunoblotting data. We also observed a redistribution of T-Cx43 GJ plaques moving from end-to-end intercalated disks to lateral cell borders (lateralization) in MH and SH hearts (Figures 3A, white circles in MH heart are examples). Quantification data showed that the lateralization percentage significantly increased in MH (29.7±3.5%, P=0.002) and SH (33.6±1.5%, P=0.001) hearts when compared to B (10.3±1.5%) hearts (Figure 3C). Furthermore, SH hearts showed a greater extent in T-Cx43 lateralization than that shown by
MH hearts (P=0.044) (Figures 3A, C). In the R hearts, the T-Cx43 lateralization percentage (24.6±3.7%) remained higher than that in B hearts (P=0.005) (Figure 3C).

Effects of MH and SH on NP-Cx43 Expression and Distribution

**NP-Cx43 Expression** We observed that NP-Cx43 levels progressively decreased in MH (by 59%, P=0.002) and SH (by 79%, P<0.001) hearts compared to B (Figures 4A, B) hearts, which is consistent with the finding that the P0 band density of T-Cx43 decreased in MH and SH hearts (Figure 2A). The difference in NP-Cx43 level between MH and SH hearts was also significant (P=0.028) (Figure 4B). In the R hearts, the NP-Cx43 level was restored to comparable baseline level (78% of the B hearts, P=0.223), and was higher than that in SH hearts (P=0.003) (Figure 4B). NP-Cx43 expression among the 4 sites was similar in B (P=0.565) and R (P=0.249) hearts (Figure 4C). However, the spatial heterogeneities in NP-Cx43 levels became significantly different in MH (P=0.017) and SH (P=0.046) hearts (Figure 4C).

**NP-Cx43 Distribution** A total of 470 areas (B: 146, MH: 96, SH: 147, R: 81 images) from 4 ventricular sites were selected for morphometric analysis. The percentage of total tissue area occupied by NP-Cx43 significantly decreased in MH (4.1±0.7%, P=0.004) and SH (3.8±1.5%, P=0.002) hearts, when compared to B (6.7±1.1%) hearts (Figures 5A, B). In R hearts, the NP-Cx43 area (4.5±0.8%) remained lower than that in B hearts (P=0.004) (Figures 5A, B). Immunofluorescent images showed that NP-Cx43 GJ lateralization was also enhanced in MH and SH hearts (Figure 5A, white circles in MH heart are examples). The lateralization percentage significantly increased in MH (30.1±1.8%, P=0.004) and SH (29.5±4.7%, P=0.002) hearts when compared to B (11.7±2.6%) hearts (Figures 5A–C). The difference between MH and SH hearts was not significant (P=0.807). In R hearts, the NP-Cx43 lateralization percentage (26.7±3.5%) remained higher than that in B hearts (P=0.004) (Figures 5A, C).

Effects of MH and SH on the Subcellular Distribution of T- and NP-Cx43 GJs

Subcellular fractionation was performed in 12 hearts (B, hearts 4, 5, 8, 9; MH, hearts 2, 3, 4, 6; SH, hearts 2, 5, 6, 8).

**Membranous Fraction** Figure 6A is an example showing the NP-Cx43 level in the membranous fraction of B, MH, and SH hearts. Compared with B hearts, the NP-Cx43 did not significantly change in MH (P=0.083) hearts (Figure 6B). However, SH hearts showed a marked decrease in NP-Cx43 level (by 77%, P=0.043) (Figures 6A, B). The NP-Cx43 levels among the 4 ventricular sites were similar in B (P=0.912), MH (P=0.290), and SH (P=0.731) hearts (Figure 6C). T-Cx43 levels in membranous fraction were unchanged in MH (P=1.0) and SH (P=1.0) hearts when compared with that in B hearts (data not shown). T-Cx43 levels among the 4 sites were also similar in B (P=0.887), MH (P=0.546), and SH (P=0.226) hearts (data not shown).

**Cytosolic Fraction** NP-Cx43 levels in cytosolic fractions were similar in MH (P=0.289) and SH (P=0.480) hearts when compared with those in B hearts (data not shown). Similarly, T-Cx43 levels were not different in MH (P=0.564) and SH (P=1.0) hearts, comparing to B hearts (data not shown). The cytosolic T- (B, P=0.936; MH, P=0.481; SH, P=0.235) and

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**Figure 6.** (A) Representative immunoblotting of the membrane fraction of NP-Cx43 levels from the 4 ventricular sites in B (heart 9), MH (heart 6), and SH (heart 2) hearts. (B) NP-Cx43 levels in the membranous fraction in these 3 groups. (C) NP-Cx43 levels in the membranous fraction at the 4 sites in B, MH, and SH hearts. a.u., arbitrary unit. See text for other abbreviations.
Therapeutic Hypothermia and Gap Junction Remodeling

Figure 7. (A) Representative immunoconfocal images of T-Cx43 GJs in SH hearts without (Left panel, heart 2, LVA) or with PKCI pretreatment (Middle panel, heart 3, LVP). (Right panel) Percentage of lateralized T-Cx43 area. (B) Representative images of NP-Cx43 GJs in SH hearts without (Left panel, heart 3, LVA) or with PKCI pretreatment (Middle panel, heart 2, LVP). (Right panel) Percentage of lateralized NP-Cx43 area. See text for abbreviations.

Figure 8. (A) Representative immunoblotting of ventricular Na\textsubscript{v}1.5 protein expression in B (heart 6) and SH (heart 8) hearts of protocol I. (B) Immunoblotting of cytosolic Na\textsubscript{v}1.5 protein in B (heart 2) and SH (heart 3) hearts of protocol III. (C) Immunoblotting of membranous Na\textsubscript{v}1.5 protein in B (heart 2) and SH (heart 3) hearts of Protocol III. Quantified data are shown in the Lower panels. See text for abbreviations.
The Na\textsuperscript{+} effects of SH on ventricular Na\textsuperscript{+} Cx43 GJ remodeling during TH remain unclear. Cx43 is a phosphoprotein, and its conduction properties and Cx43 GJ remodeling specifically explore GJ remodeling. Decreased Cx43 dephosphorylation during TH might have little effect on the remodeling processes. The blockade of only T-Cx43 in either fraction. Changes in the phosphorylation status of Cx43 (ie, decreased NP-Cx43 during TH) could regulate the trafficking, assembly, and degradation of Cx43. PKC pathway in TH-induced Cx43 remodeling. PKC can phosphorylate the intracellular C-terminal domain of Cx43 at Ser\textsuperscript{505}, the phosphorylation of which was reported to alter channel selectivity and GJ trafficking. A previous study has shown that temperature preconditioning (hypothermia) protected hearts from ischemia-reperfusion injury by activating the PKC pathway. In this study, we found that TH caused a decrease in NP-Cx43 expression and an increase in T- and NP-Cx43 lateralization, implying that the PKC pathway might participate in TH-induced Cx43 lateralization. In Protocol II, we observed that pretreatment of a PKCI (chelerythrine) significantly attenuated SH-induced NP-Cx43 lateralization, while T-Cx43 lateralization, T- and NP-Cx43 expression were unchanged. Our data suggest that PKC is involved in SH-induced NP-Cx43 lateralization, but does not directly influence Cx43 dephosphorylation. Two possible mechanisms might explain why chelerythrine could not prevent SH-induced T-Cx43 lateralization. First, SH might activate multiple signaling pathways that were not thoroughly evaluated in this study. The blockade of only a single PKC pathway might not completely reverse SH-induced T-Cx43 lateralization. Second, NP-Cx43 normally makes up only ~10% of T-Cx43. The attenuation in NP-Cx43 lateralization by chelerythrine might have little effect on overall T-Cx43 lateralization.
global ischemia in the isolated rabbit heart caused significant T-Cx43 lateralization, while CV was unchanged, which was similar to our findings. Therefore, NP- and T-Cx43 lateralization might not play a major role in CV slowing during TH in this acute model.

Spatial Heterogeneity of CVRs During MH and SH
In previous studies, conduction heterogeneities (evaluated by anisotropic ratio) and GJ remodeling during hypothermia were characterized globally; namely, the representative area of myocardium was studied without exploring different sites of the heart.4,5,26 In this study, we investigated GJ remodeling at the exact sites where conduction disturbances happened. A novel finding in the present study was that the enhancement of spatial heterogeneity of CVRs in MH and SH hearts were associated with (1) heterogeneous reduction in NP-Cx43 level and (2) increased NP-Cx43 lateralization. Although rearming the hearts could abolish the spatial heterogeneity of CVRs and restore NP-Cx43 expression, NP-Cx43 lateralization did not recover. Therefore, the individual contribution of NP-Cx43 remodeling to TH-induced heterogeneous conduction remains unclear.

Contribution of Cx43 GJ Remodeling to Conduction Disturbance During TH
In this acute model (short-duration TH and rearming), GJ remodeling might not be the major determinant of conduction disturbance during TH. Two possible mechanisms might explain this notion. First, previous studies from Cx43 gene knockdown mice showed that >50% reduction in T-Cx43 expression is essential to cause significant CV slowing.30 In contrast, T-Cx43 levels were unchanged during TH in the present study. Second, in addition to GJ coupling, myocardial conduction is also determined by fast inward Na+ current (tissue excitability) and extracellular matrix (fibrosis).11-13 We used healthy hearts in which myocardial fibrosis was probably not altered by temperature. Furthermore, Na+1.5 protein expression and internalization were not influenced by short-duration SH. These findings support the idea that functional rather than structural changes in the Na+1.5 channel may occur soon after induction of short-duration TH and may play a major role in conduction disturbance.11,15

Clinical Implications
SH hearts showed a greater extent of CV slowing, NP-Cx43 down-regulation, and T-Cx43 lateralization, and more hearts with inducible VF, compared to MH hearts. Therefore, 33°C might be a more feasible temperature for patients receiving TH than 30°C.2

Study Limitations
In this model, Cx43 GJ remodeling caused by short-duration (30 min) TH might not play a major role in conduction disturbance. Whether a longer duration (12–24 h) of TH (compliant with clinical guidelines) might lead to a greater extent of Cx43 GJ remodeling leading to significantly impaired ventricular conduction warrants further investigation.

Conclusion
Short-duration (30 min) TH causes a prompt, temperature-dependent Cx43 GJ remodeling, in which the PKC pathway is involved. However, GJ remodeling alone might not play a major role in the conduction disturbance of this acute model.

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