Rapid Electrical Stimulation of Contraction Modulates Gap Junction Protein in Neonatal Rat Cultured Cardiomyocytes

Involvement of Mitogen-Activated Protein Kinases and Effects of Angiotensin II-Receptor Antagonist

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OBJECTIVES	The aim of this study was to investigate the effects of rapid electrical stimulation (RES) of contraction on the expression of connexin (Cx)43 gap junction in neonatal rat cultured ventricular myocytes and the consequent changes of conduction properties.
BACKGROUND	The expression and distribution of gap junctions in cardiac muscle can be changed readily under a variety of pathological conditions because of dynamic turnover of Cxs. The effects of RES of contraction on gap junction remodeling are not well understood.
METHODS	Neonatal rat ventricular myocytes cultured for five days were subjected to RES (field stimulation) at 3.0 Hz for up to 120 min.
RESULTS	Rapid electrical stimulation resulted in a significant upregulation of Cx43 (by ~1.5-fold in protein and by ~1.9-fold in messenger ribonucleic acid at 60 min). Immunoreactive signal of Cx43 was also increased. Angiotensin II (AngII) content was increased significantly by RES >15 min. Phosphorylated forms of extracellular signal-regulated protein kinase (ERK), c-Jun NH$_2$-terminal kinases, and p38 mitogen-activated protein kinases (MAPKs) were all increased dramatically by RES with peaks at 5 ~ 60 min. Propagation of excitation was visualized by extracellular potential mapping by using a multiple electrode array system. Conduction velocity was increased significantly by RES for 60 to 90 min (25% ~ 27% increase). Treatment of myocytes with losartan (100 nmol/l) prevented most of these effects of RES; RES-induced upregulation of Cx43 was also prevented by specific inhibitors for ERK and p38 MAPKs.
CONCLUSIONS	A short-term RES causes upregulation of Cx43 in cardiomyocytes and a concomitant increase of conduction velocity, mainly through an autocrine action of AngII to activate ERK and p38 MAPKs. (J Am Coll Cardiol 2004;44:914–22) © 2004 by the American College of Cardiology Foundation

Cardiac myocytes are connected with each other electrically and metabolically through gap junction channels composed of connexins. Many animal and human studies have described alterations in the abundance or subcellular localization of connexin proteins under a variety of pathological conditions, including hypertrophy, heart failure, ischemic heart diseases, and atrial fibrillation (1,2). Many connexins, including connexin43 (Cx43), which is expressed most abundantly in the heart, have short half-lives of 1 ~ 5 h, reflecting their rapid turnover through synthesis and degradation (3–5).

Zhuang et al. (6) demonstrated in monolayers of rat cultured ventricular myocytes that application of linear pulsatile stretch (10% resting length at 3 Hz) only for 1 h caused an increase of Cx43 expression by ~2-fold, and the change was associated with a significant increase of conduction velocity. If the mechanical stretch on the myocytes plays a primary role in the upregulation of Cx43, an increase of twitch contraction per unit of time resulting from a sudden increase of heart rate is expected to cause a comparable mechanical stimuli to affect the expression of gap junctions. The present study was designed to test this hypothesis.

We investigated the effects of rapid electrical stimulation (RES) on the expression of Cx43 gap junction in neonatal rat cultured ventricular myocytes and the consequent changes of conduction properties. As to the signaling cascade, we focused on the role of angiotensin II (AngII) and mitogen-activated protein kinase (MAPK) family because AngII is known to be secreted from cardiac myocytes that have been subjected to mechanical stretch and to play a crucial role in autocrine action in stretch-induced hypertrophic responses through activation of multiple kinases, in-

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Abbreviations and Acronyms

AngII	=	angiotensin II
Cx43	=	connexin43
ERK	=	extracellular signal-regulated protein kinases
JNK	=	c-Jun NH$_2$-terminal kinases
MAPK	=	mitogen-activated protein kinase
mRNA	=	messenger ribonucleic acid
RES	=	rapid electrical stimulation

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cluding MAPKs (7–9). The results have first revealed that a short-term RES of contraction causes a prominent increase of Cx43 expression (messenger ribonucleic acid [mRNA] and protein) in cardiac myocytes, mainly through autocrine action of AngII to activate MAPKs, and these observations are associated with substantial alterations of conduction properties.

METHODS

All the animals were handled according to the guideline of the Animal Care Committee of Yamaguchi University. This investigation complied with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Cell culture. Primary cultures of one- to two-day-old neonatal rat ventricular myocytes were prepared as reported previously (10). The myocytes dissociated enzymatically were seeded on culture trays at a concentration of 3×10^5 cells/cm^2, and then incubated in Leibovitz L-15 Medium (Worthington Biochemical Co., Lakewood, New Jersey) supplemented with 5% fetal bovine serum at 37°C. The cell suspension was replated to reduce the fibroblast content. Cells were grown in a random orientation (isotropic growth) to make a confluent layer, and by day 5 they showed synchronized spontaneous beating with a regular frequency. The relative population of myocytes over nonmyocytes in the respective culture, that was estimated by immunolabeling against antiactin antibody, was >97%.

RES of contraction. Cultured myocytes on day 5 were transferred to serum-free medium, and subjected to RES at 3.0 Hz for up to 120 min; RES was applied by field stimulation via platinum wire electrodes (0.5 mm in diameter) with an interpolar distance of 6.5 cm (for experiments of Cx43 expression analysis) or of 0.5 cm (for potential mapping experiments). The stimulation intensity was adjusted to a level twice the threshold for synchronous contraction. The pulse polarity was alternated one after another to minimize the electrolysis and possible generation of oxidative species. Ascorbic acid (250 μmol/l), a scavenger of oxidative species, was added to the culture medium (11). In experiments to elucidate the signaling cascade, we used a specific AngII type-1 receptor antagonist, losartan (gift from Merck & Co. Inc., Rahway, New Jersey), and specific MAPK inhibitors (PD98059, SP600125, and SB203580) (Calbiochem, San Diego, California). These compounds at appropriate concentrations were applied to the culture medium 30 min before the initiation of RES.

Extracellular potential mapping of propagation. Conduction properties of the cultured ventricular myocytes were examined by extracellular potential mapping by using a specifically designed multielectrode array system (MED-P545A and MED64, Panasonic, Tokyo, Japan) (12,13). The 64 planar microelectrodes, each having a size of 50 × 50 μm, were arranged in an 8 by 8 pattern with an interelectrode distance at 450 μm to cover a 3,150 × 3,150 μm area beneath the cultured myocytes. All of the 64 unipolar signals were recorded simultaneously with a common reference from four distant electrodes, and amplified with high gain (60 dB) and at a low frequency (1.0 ~ 100 Hz) filter setting. Constant stimuli at 3.0 Hz were applied via four lateral electrodes of the recording array to elicit one-way propagation of excitation in the observation area before and after RES. Biphasic pulses of 0.1 ms duration were employed for the stimulation with an intensity of 100 to 200 μA (twice the diastolic threshold). Activation time at each recording site was identified by the sharp negative deflection. The conduction velocity was measured by plotting activation time against distance from the stimulation site along a midline from left to right.

Electrophoresis and immunoblotting. Collected cells were lysed, and the cellular extracts were electrophoresed on SDS-polyacrylamide gels. The following primary antibodies were used for immunodetection: rabbit anti-connexin43 antibody (Zymed, South San Francisco, California); goat anti-angiotensin I/II antibody (Santa Cruz, Santa Cruz, California); rabbit anti-extracellular signal-regulated protein kinases (ERK)1/2 antibody; rabbit anti-c-Jun NH$_2$-terminal kinases (JNK)1 antibody; rabbit anti-p38 antibody (Santa Cruz, California); rabbit anti-active MAPK pAb; rabbit anti-active JNK pAb; rabbit anti-active p38 pAb (Promega, Madison, Wisconsin). The amount of protein recognized by the antibodies was quantified by means of an ECL immunoblotting detection system (Amersham, Buckinghamshire, England).

Reverse transcription and polymerase chain reaction amplification. Total cellular ribonucleic acid was isolated from each frozen cell sample by the acid guanidinium thiocyanate/phenol/chloroform extraction method (14). Complementary deoxyribonucleic acid was prepared using a Takara RNA PCR Kit (Takara, Tokyo, Japan) (15). The primers for the amplification of Cx43 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were designed from published sequences. For Cx43, they were based on rat sequences (GenBank, accession no. P08050) (16): at positions 367 to 390 (sense primer, 5′-GTG GAG ATG CAC CTG AAG CAG ATT-3′) and 1,038 to 1,058 (antisense primer, 5′-AGT TGC TGC TGG ACA TGA ACT-3′) (predicted length of the PCR product, 671 base pairs [bp]). For GAPDH, they were based on rat sequences: at positions 102 to 125 (sense primer, 5′-CTT CAT TGA CCT CAA CTA CAT GGT-3′) and 805 to 828 (antisense primer,
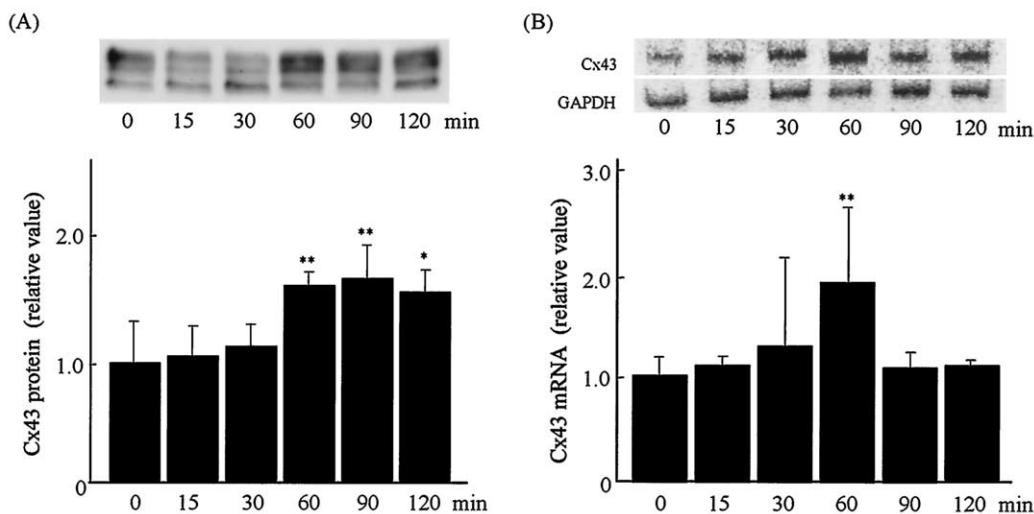
Figure 1. Quantitative analysis of connexin (Cx)43 protein and messenger ribonucleic acid (mRNA) levels in cardiomyocytes subjected to rapid electrical stimulation. **(A)** Connexin43 protein levels estimated by Western analysis; Cx43 amount was normalized to control protein (mean ± SD, n = 8). *p < 0.05; **p < 0.01 vs. baseline. **(B)** Connexin43 mRNA levels estimated by reverse transcription-polymerase chain reaction. Connexin43 mRNA amount was normalized to GAPDH (means ± SD, n = 8). **p < 0.01 vs. baseline.

5′-CTC AGT GTA GCC CAG GAT GCC CTT-3′) (predicted length of the PCR product, 726 bp). The GAPDH gene product was used as an internal control.

Cell size measurement and immunohistochemistry. Cardiomyocytes on culture slides were fixed and stained with hematoxylin-eosin for measurements of their dimension. The cell area of myocytes was measured in 10 random fields (50 cells/field) with the aid of NIH Image (Bethesda, Maryland).

For immunodetection of gap junctions, a polyclonal rabbit anti-Cx43 antibody (Zymed, California) was used. After permeabilization (0.1% Triton X-100), quenching and blocking (10% goat serum), samples were incubated with the antibody (1:100 diluted in PBS) overnight at room temperature. Primary antibody-bound Cx43 was visualized by FITC-conjugated anti-rabbit IgG, and examined using a confocal microscope (LSM500, Zeiss, Germany). Samples processed without primary antibody served as negative control. The proportion of Cx43 immunoreactive signal was defined as the number of high signal intensity (>90% of the maximum level) pixels divided by total number of pixels occupied by the myocytes (6). The 10 individual measurements at different fields were averaged to yield a single value for each culture slide.

Measurement of AngII concentration. Angiotensin II contents in culture media under the respective condition were measured by radioimmunoassay as previously described (17).

Statistical analysis. All data are presented as the mean ± SD. Differences in continuously distributed variables between predetermined experimental groups were analyzed using one-way analysis of variance followed by Dunnett's multiple comparison method with SPSS software (SPSS Inc., Chicago, Illinois). We did not model experimental

design as repeated measures. Differences were taken to be significant at p < 0.05.

RESULTS

Effects of RES on cell size and spontaneous beating rate of cultured cardiomyocytes. Cell size was measured in each eight culture slides. Cell area at the baseline was 610 ± 141 μm^2. The values at 15, 30, 60, 90, and 120 min after RES were 620 ± 134, 570 ± 119, 594 ± 124, 626 ± 138, and 547 ± 91 μm^2, respectively, and they were not significantly different from the baseline. There were no appreciable differences in the cell density among the groups. The baseline spontaneous beating rate was 130 ± 10 beats/min (n = 8). Rapid electrical stimulation for 15 to 120 min had no significant effects on the spontaneous beating rate.

Effects of RES on Cx43 expression. In the immunoblotting (Fig. 1A), the Cx43 antibody recognized three bands migrating between 40 and 43 kDa. The amount of Cx43 protein normalized to the value for control protein was increased gradually by RES, and the change reached a statistical significance at 60 min (by 1.53 ± 0.11-fold, n = 8, p < 0.01). The amount of Cx43 mRNA was also increased by RES up to 60 min (by 1.90 ± 0.73-fold, n = 8, p < 0.01) (Fig. 1B). The increase of Cx43 protein reached a peak at 90 min, and then began to decline. The mRNA returned to the baseline already at 90 min.

Figure 2A shows confocal images of Cx43 immunolabeling. Connexin43-containing gap junctions were visualized as aggregates of bright punctate fluorescent domains around the cell perimeter at the abutment with neighboring cells. Rapid electrical stimulation for 60 to 90 min resulted in a homogeneous increase of the Cx43 immunoreactive signals without affecting their distribution pattern. Quantitative
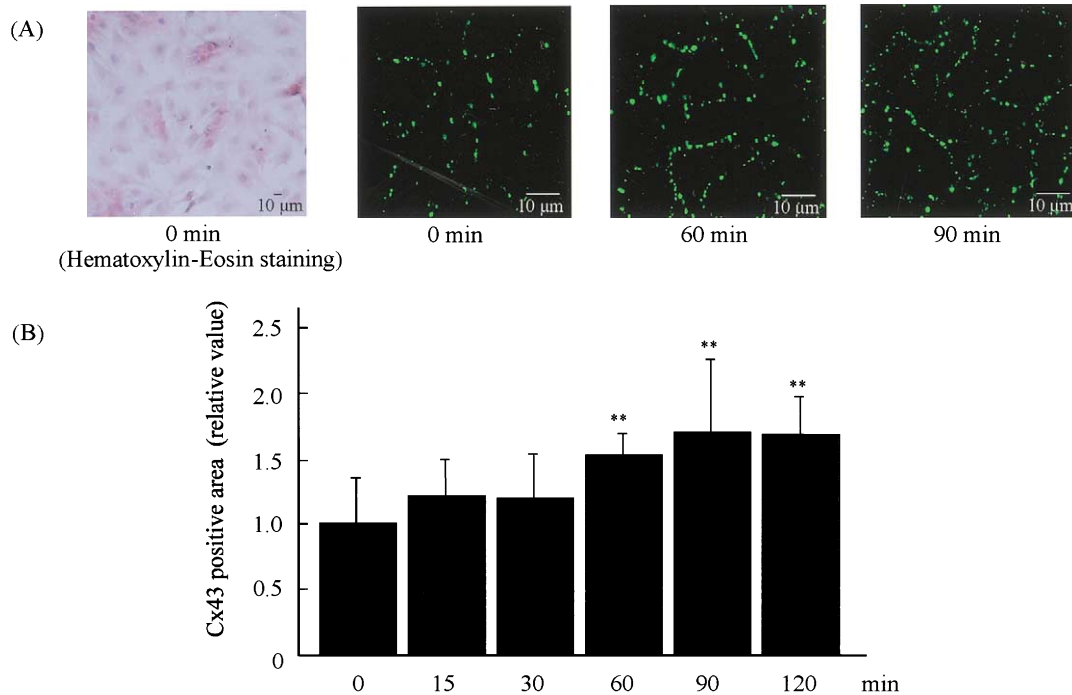
(A)

0 min
(Hematoxylin-Eosin staining)

0 min

60 min

90 min

(B)

Figure 2. Confocal images of connexin (Cx)43 immunolabeling in cultured cardiomyocytes. **(A)** A picuture of cultured cardiomyocytes and representative immunofluorescence images of Cx43 before (0 min) and after rapid electrical stimulation for 60 min and 90 min. **(B)** The proportion of total cell area occupied by Cx43 immunoreactive signal (means $\pm$ SD, n = 8). **p < 0.01 vs. 0 min (baseline).

analysis revealed a significant increase in the Cx43-positive area by RES (by 1.53 ± 0.18-fold at 60 min, and 1.71 ± 0.55-fold at 90 min) (Fig. 2B).

Effects of RES on AngII production and secretion by cardiomyocytes. We examined the effect of RES on AngII content in the culture media and cardiomyocytes. The AngII content in the culture media was increased remarkably by RES for 15 min, and the elevation was maintained thereafter (Fig. 3A). Figure 3B shows the protein amount of AngII in cardiomyocytes estimated by immunoblotting. Angiotensin II was increased significantly after RES for 15 to 60 min (by $1.6 \sim 1.9$-fold). Further prolongation of RES was associated with a gradual return of the AngII amount toward the baseline. These observations indicate rapid increases in production and secretion of AngII by cardiomyocytes subjected to RES.

Effects of RES on ERK, JNK, and p38 MAPK. We next examined three major MAPK signaling pathways downstream of AngII. The total ERK level was increased significantly by RES for 5 min (by 1.6-fold) (Fig. 4A). Phosphorylated ERK (p-ERK) showed a much greater increase in response to RES with dual peaks at 5 min (by 2.1 ± 1.1-fold, $p < 0.05$) and at 60 min (by 3.2 ± 0.8-fold, $p < 0.01$) (Fig. 4A). Although the total JNK level was unaffected, phosphorylated form of JNK (p-JNK) was increased dramatically in response to RES with dual peaks at 15 min (by 3.5 ± 1.6-fold, $p < 0.01$) and at 60 min (by 4.1 ± 1.3-fold, $p < 0.01$) (Fig. 4B). The total p38 MAPKs (total p38) was unchanged, but phosphorylated form of p38 MAPKs (p-p38) showed the most pronounced and rapid

increase in response to RES with a single peak at 5 min (by 6.5 ± 1.0-fold, $p < 0.01$) (Fig. 4C). These results suggest that MAPK signaling pathways must be activated by RES.

(A)Table. Angiotensin II Content of Culture Media

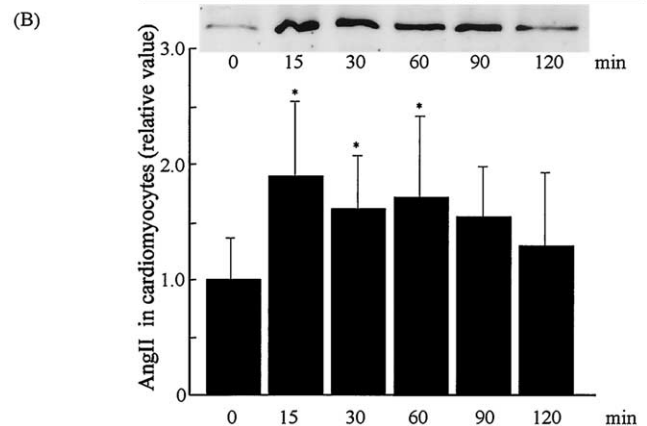|	Angiotensin II (pg/ml)
baseline	< 3
15 min	16.8 $\pm$ 3.6 **
30 min	20.0 $\pm$ 2.6 **
60 min	19.0 $\pm$ 2.8 **
90 min	19.4 $\pm$ 2.8 **

(B)

Figure 3. Angiotensin II (AngII) secretion and production by cultured cardiomyocytes. **(A)** Angiotensin II content in the culture media (means $\pm$ SD, n = 8). **p < 0.01 vs. 0 min (baseline). **(B)** Angiotensin II content of cardiomyocytes (Western analysis). Values were normalized to the baseline (mean $\pm$ SD, n = 8). *p < 0.05 vs. baseline.
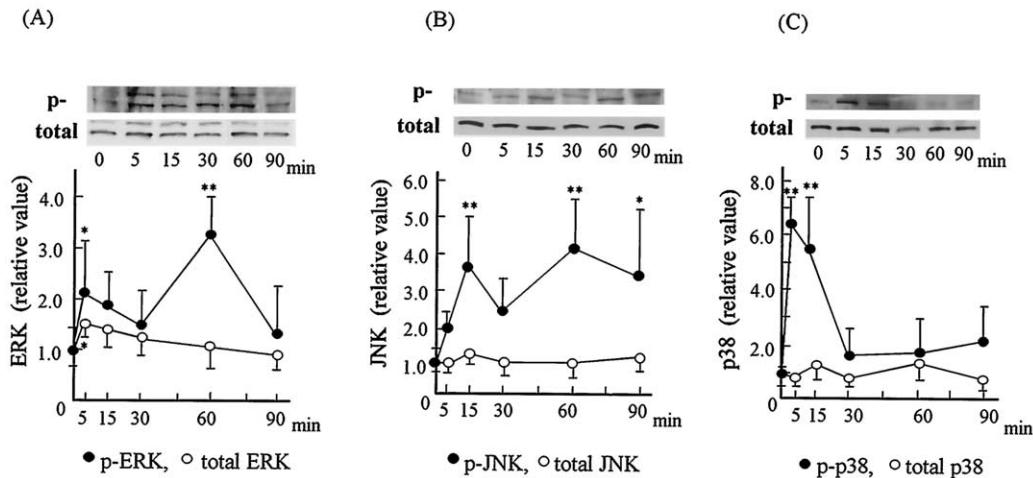
Figure 4. Changes of three mitogen-activated protein kinases (MAPKs) and their phosphorylated forms in response to rapid electrical stimulation (Western analysis). **(A)** Total extracellular signal-regulated protein kinases (ERK) and phospho-ERK (p-ERK). **(B)** Total c-Jun NH$_2$-terminal kinases (JNK) and phospho-JNK (p-JNK). **(C)** Total p38 MAPKs and phospho-p-38 MAPKs (p-p38). Values were normalized to their baseline (mean ± SD, n = 8). *p < 0.05; **p < 0.01 vs. baseline.

Prevention of RES-induced Cx43 upregulation and MAPK activation by losartan. If RES-induced Cx43 upregulation is the result of autocrine action of AngII to activate MAPKs, the increase of Cx43 expression and the level of activated forms of MAPKs should be prevented by pharmacologic blockade of AngII type 1 receptor. Figure 5 shows the effects of RES on the Cx43 expression in the presence of losartan. The baseline protein and mRNA levels
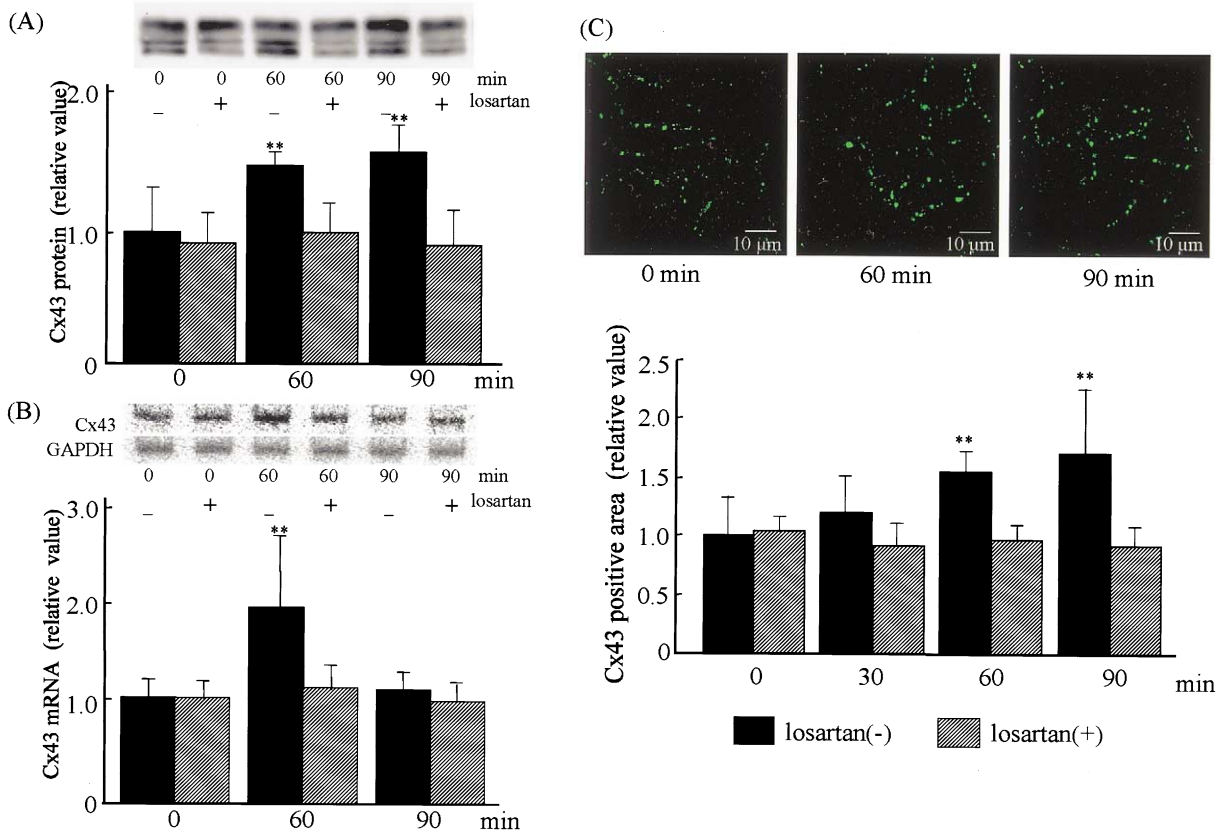

Figure 5. Prevention of rapid electrical stimulation (RES)-induced connexin (Cx)43 upregulation by losartan. **(A)** Connexin43 protein levels before (0 min) and after RES for 60 min and 90 min in the absence **(solid bars)** and presence **(hatched bars)** of 100 nmol/l losartan (Western analysis). Connexin43 amount was normalized to control protein (means ± SD, n = 8). **(B)** Connexin43 messenger ribonucleic acid (mRNA) levels before (0 min) and after RES for 60 min and 90 min (reverse transcription-polymerase chain reaction). Connexin43 mRNA amount was normalized to GAPDH (mean ± SD, n = 8). **(C)** Immunofluorescence signals of Cx43 before (0 min) and after RES for 30 to 90 min. **(Top)** Representative confocal images. **(Bottom)** The proportion of total cell area occupied by Cx43 immunoreactive signal. Values were normalized to baseline (mean ± SD, n = 8). **p < 0.01 vs. baseline.
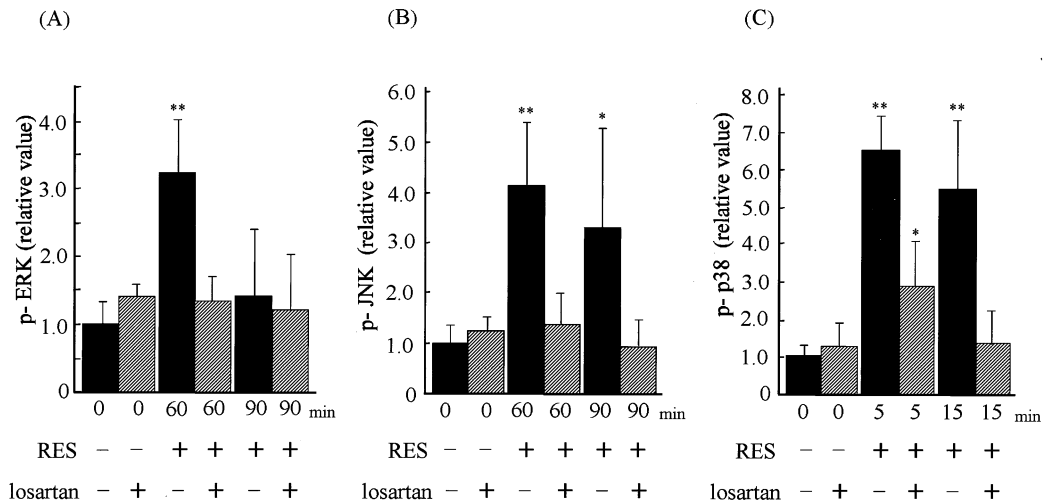
(A)   (B)   (C)

Figure 6. Prevention of rapid electrical stimulation (RES)-induced activation of mitogen-activated protein kinase (MAPK) by losartan. **(A)** Phospho-extracellular signal-regulated protein kinases (ERK) (p-ERK) levels before (0 min) and after RES for 60 min and 90 min in the absence **(solid bars)** and presence **(hatched bars)** of 100 nmol/l losartan. **(B)** Phospho-c-Jun NH$_2$-terminal kinases (JNK) (p-JNK) levels. **(C)** Phospho-p38 MAPKs (p-p38) levels. The amount of each protein was normalized to the baseline without losartan (mean ± SD, n = 8). *p < 0.05; **p < 0.01 vs. baseline.

of Cx43 were unaffected by treatment of the cardiomyocytes with losartan (100 nmol/l). In the presence of losartan, unlike in the absence of compound, RES for 60 or 90 min did not cause significant increases in protein (Fig. 5A) and mRNA (Fig. 5B) of Cx43. In the immunohistochemistry, RES for 30 to 90 min in the presence of losartan did not cause significant changes in the Cx43-positive area (Fig. 5C).

Figure 6 shows the effects of RES on the activated forms of MAPKs. In the presence of losartan, RES for 60 to 90 min did not cause significant increase of p-ERK and p-JNK. The level of p-p38 was increased by RES for 5 min even in the presence of losartan (by 2.9 ± 1.1-fold, p < 0.05), but the increases were much less than that in the absence of losartan. The p-p38 response to RES for 15 min was abolished by losartan. These results indicate effective prevention of RES-induced Cx43 upregulation and MAPK activation by AngII type 1 receptor blockade.

To examine the relative contribution of different MAPKs, RES was applied to cardiomyocytes pretreated with specific inhibitors for each branch of the MAPKs (Fig. 7). The baseline protein level of Cx43 was unaffected by either PD98059 (50 μmol/l), SP600125 (50 μmol/l), or SB203580 (10 μmol/l). In the presence of PD98059, an inhibitor of MAPK/ERK, the RES-induced increase of Cx43 protein was almost abolished. Pretreatment with SP600125, a selective inhibitor of JNK pathway, in contrast, did not affect the RES-induced Cx43 upregulation. Pretreatment with SB203580, an inhibitor of p38 MAPKs and JNK2 pathway caused a partial prevention of the Cx43 upregulation. These observations suggest that the RES-induced Cx43 upregulation is mediated mainly by an activation of ERK and p38 MAPK pathways.

Effects of RES on the propagation of excitation. We investigated functional manifestation by multielectrode ex-

tracellular potential mapping. The measurements were made before and after RES. A clear local electrogram composed of a positive deflection followed by negative deflection was recorded from most (>90%) of the 64 electrodes (Fig. 8A). Isochrone maps of activation time showed almost uniform propagation of excitation from left to right both before (control) (Fig. 8B) and after RES for 90 min (Fig. 8C). The activation times after RES at the respective recording sites were much shorter than those of control, indicating the RES-induced acceleration of propagation. Such changes in propagation after RES were prevented by pretreatment with losartan (100 nmol/l) (Fig. 8D). Figure 9 shows the pooled data of conduction velocity.
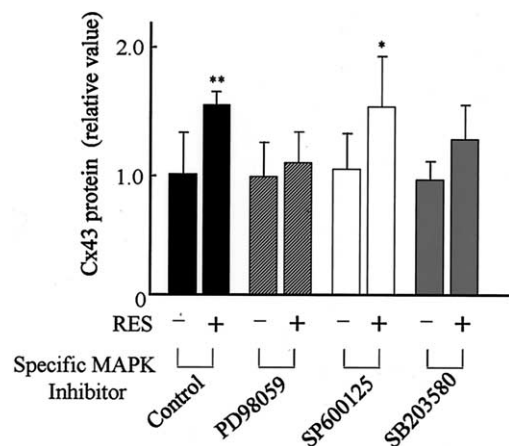

Figure 7. Prevention of rapid electrical stimulation (RES)-induced upregulation of connexin (Cx)43 by specific mitogen-activated protein kinase (MAPK) inhibitors. Connexin43 proteins levels before (0 min) and after RES for 60 min in the absence and presence of 50 μmol/l PD98059 (a MAPK/extracellular signal-regulated protein kinase inhibitor), 50 μmol/l SP600125 (a selective c-Jun NH$_2$-terminal kinases [JNK] inhibitor), and 10 μmol/l SB203580 (a p38 and JNK2 inhibitor). Values were normalized to baseline in the control medium (mean ± SD, n = 8). *p < 0.05; **p < 0.01 vs. baseline.

(A) extracellular potentials

(B) control

3150µm

(C) RES 90min without losartan
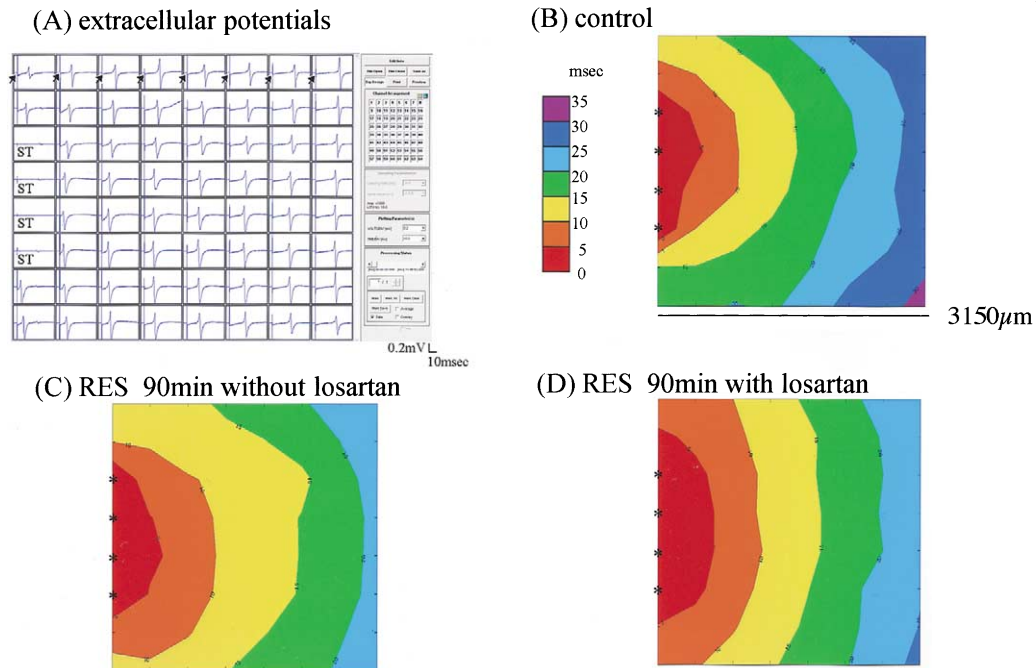
(D) RES 90min with losartan

Figure 8. Multielectrode extracellular potential mapping during propagation of excitation. **(A)** Representative electrograms recorded from the 64 terminals. **(Arrows)** Stimulus artifacts. **(B and C)** Isochrone maps of activation time in a culture dish before (control) and after rapid electrical stimulation (RES) for 90 min in the absence of losartan. **(D)** Isochrone map of activation time in another culture dish after RES for 90 min in the presence of 100 nM losartan. ST = stimulation point; * = stimulation points.

In the absence of losartan, the conduction velocity was increased by RES >30 min; the values at 60 min and at 90 min after RES were significantly higher than control. In the presence of losartan, RES did not affect the conduction velocity.

DISCUSSION

In the present study, we showed that a short-term RES of contraction caused a prominent increase of Cx43 expression in cardiac myocytes. This was associated with an increase of AngII production and secretion by the myocytes as well as upregulation of activated forms of MAPKs. Parallel elevation of mRNA and protein suggest that an increase of synthesis may play an important role in the upregulation of
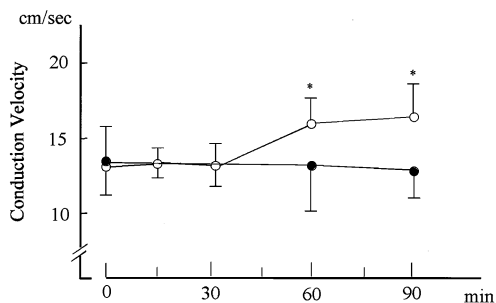

Figure 9. Effects of rapid electrical stimulation (RES) on the conduction velocity. Conduction velocity from **left to right** was measured along the midline in the observation area before and 15 to 90 min after RES. Values (means ± SD) obtained in the absence (**open circle**, n = 7 to 9) and presence of 100 nM losartan (**solid circle**, n = 7 to 9) were plotted against time after the initiation of RES. *p < 0.05 vs. baseline value before RES.

Cx43. Extracellular potential mapping revealed an acceleration of propagation by RES. Most of these effects of RES were prevented by losartan, an AT1 blocker.

Signaling pathway for the RES-induced Cx43 upregulation. In cultured neonatal rat cardiomyocytes, several MAPK family members were shown to be activated in response to pulsatile stretch (9). Extracellular signal-regulated protein kinases, JNK, and p38 are activated by G-protein-coupled receptor agonists such as AngII or endothelin-1 (18). Extracellular signal-regulated protein kinases, JNK, and p38 can also be activated by a variety of cellular stress such as hyperosmotic shock, protein synthesis inhibitors, hypoxia/reoxygenation, and reactive oxygen species (18). Our results have revealed that RES does cause prompt and dramatic increases of the phosphorylated forms of ERK, JNK, and p38. In addition, most of the RES-induced activation of these MAPKs was prevented by losartan. The RES-induced upregulation of Cx43 protein was also prevented by the inhibitors of ERK and p38 MAPKs (PD98059 and SB203580), whereas it was unaffected by the inhibitor of JNK (SP600125). These observations seem to suggest pivotal roles of ERK and p38 MAPKs in the downstream of AngII signaling pathways. Polontchouk et al. (19) have demonstrated that application of exogenous AngII to cultured rat cardiomyoctes causes a specific upregulation of Cx43 within 24 h through signaling pathways including activation of ERK1/2 and p38. The longer time required for Cx43 upregulation might be due to less efficiency of exogenous AngII than its autocrine action in stimulating the downstream pathways.

We cannot exclude mediators other than AngII for the upregulation of Cx43, because the early activation of p38 by RES was not completely prevented by losartan (Fig. 6C). Those might include cAMP (3), Wnt-1/β-catenine (20), and vascular endothelial growth factor (21). More extensive experimental studies will be required to elucidate the exact signaling pathway for the RES-induced Cx43 upregulation.

Functional manifestation of the RES-induced Cx43 upregulation. The present data demonstrate that RES for 60 to 90 min causes a significant increase of the conduction velocity. If it is assumed that the increase in Cx43 immunoreactive signal (1.53- to 1.71-fold after 60 to 90 min RES) is proportional to the increase in gap junctional conductance, the increase of conduction velocity (25% to 27%) is attributed most likely to an upregulation of gap junction channels.

Several other possible mechanisms, however, should also be taken into account for the altered conduction properties after RES. Newly synthesized Cx43 gap junctions could have different functions than those of basal condition. Yao et al. (22) reported recently that remodeled gap junction channels in a border zone of healing infarcts had lower conductance and altered voltage-dependence. Potential upregulation of other connexins including Cx45 (5) remains to be investigated. Post-translational changes in Cx43 may also affect the electrical coupling through gap junctions. Connexin43 can be phosphorylated by protein kinase A, C, G, and mitogen-activated protein kinase, and the sarcoma protein kinase (5,23). Actions of these kinases usually lead to diminished or increased single-channel or whole-cell conductances, but the responsible mechanisms are poorly defined (5,23). Phosphorylation is also involved in the connexin stability to form gap junction channels. Increasing evidence has focused attention on this process in remodeling of gap junctions in diseased hearts (5,23). Recently, Beardslee et al. (24) showed in a model of acute ischemia that uncoupling of cardiac tissue is associated with dephosphorylation of Cx43 and accumulation of unphosphorylated Cx43 in the gap junction plaques. Rapid electrical stimulation of contraction might affect the conduction properties through a modulation of Cx43 phosphorylation, and it will be an interesting issue to be studied in the future.

Impulse propagation in cardiac tissue is generally assumed to be impaired by a reduction of intercellular electrical coupling. If the tissue contains cellular discontinuities, however, the modification is more complex. Rohr et al. (25) have demonstrated in patterned growth myocyte culture that partial cellular uncoupling causes paradoxical improvement of impulse conduction at the site of abrupt geometric expansion. Accordingly, an increase in electrical cell-to-cell coupling by RES could jeopardize conduction at the corresponding sites in the heart, leading to unidirectional conduction block and reentrant arrhythmias. Proarrhythmic propensity after tachycardia might be attributed, in part, to such altered conduction properties.

Conclusions. The present study has first revealed that a short-term RES of contraction causes a prominent increase of Cx43 expression, which is mediated mainly by AngII-MAPKs pathways, and it is accompanied by alterations of conduction properties.

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