Transcription Enhancer Factor-1–Related Factor–Transgenic Mice Develop Cardiac Conduction Defects Associated With Altered Connexin Phosphorylation

Hsiao-Huei Chen, PhD; Catherine J. Baty, DVM, PhD; Tomoji Maeda, PhD; Steven Brooks, MD; Linda C. Baker, PhD; Takahisa Ueyama, MD, PhD; Erdal Gursoy, MD, PhD; Samir Saba, MD; Guy Salama, PhD; Barry London, MD, PhD; Alexandre F.R. Stewart, PhD

Background—Conduction system defects and slowed ventricular conduction are common in patients with systolic dysfunction and contribute to arrhythmias and sudden death. In animal models of heart failure, cardiac α₁-adrenergic signaling is constitutively activated. Here, we report the effects of constitutive activation of α₁-adrenergic signaling on connexin phosphorylation and cardiac conduction.

Methods and Results—Transgenic mice were generated with cardiac-specific overexpression of the transcription factor RTEF-1 (transcription enhancer factor-1–related factor), which mediates α₁-adrenergic signaling in cardiac myocytes. Surface and intracardiac ECGs revealed prolongation of the PR, QRS, and AH intervals and the appearance of progressive atrial arrhythmias in RTEF-1 mice. Optical mapping using voltage-sensitive dye revealed slower conduction velocities across the atrial and ventricular myocardium. Intercellular dye transfer between RTEF-1 transgenic cardiac myocytes confirmed impaired conduction at the cellular level. Conduction defects were correlated with dephosphorylation of connexin40 and connexin43 and upregulation of protein phosphatase 1β (PP1β). Overexpression of PP1β in HeLa cells dephosphorylated cardiac connexin. Confocal microscopy revealed increased levels of dephosphorylated connexin43 at the cardiac gap junctions in RTEF-1 mice, suggesting that defective conduction is a result of impaired gap-junction conductance rather than assembly.

Conclusion—Constitutive activation of α₁-adrenergic signaling through the RTEF-1 transcription factor results in chronic elevation of PP1β expression and connexin dephosphorylation. This mechanism may underlie some defects in cardiac conduction. (Circulation. 2004;110:2980-2987.)

Key Words: gap junctions ■ transcription factors ■ ventricles ■ atrium ■ arrhythmias

The molecular basis for impaired conduction associated with most heart disease is unknown. Gap junctions are the membrane channels that mediate direct electrical coupling between cardiac myocytes.1 Connexin40, 43, and 45 encode the cardiac gap-junction proteins. Connexin45 is of low abundance and is found in the ativoventricular (AV) node and adjoining His bundle. Connexin43 is the most abundant connexin in the heart and is expressed in the atrial and ventricular myocardium. Connexin40 is expressed in atrial tissue and the fast-conducting tissue of the His-Purkinje system but not in the compact AV node or in the ventricular myocytes. Reduced connexin expression or modifications that affect gap-junctional conductance could impair conduction and lead to arrhythmias.

Connexin43- and connexin45-null mutant mice die at birth or during embryonic development,2,3 and conditional deletion of cardiac connexin43 after birth causes a marked slowing of ventricular conduction and ventricular fibrillation.4 Connexin40-null mice develop AV nodal conduction delays5,6 and impaired conduction in the bundle branches.7 In addition, disrupting transcription factor function or protein processing leads to downregulated connexin expression and defects in cardiac conduction.8–11

Changes in connexin phosphorylation can also alter gap-junction conductance in vitro.12–14 Connexin phosphorylation depends on the balance between protein kinases and protein phosphatases. Protein kinase A (PKA) and protein kinase C are activated by β- and α₁-adrenergic receptors, respectively. However, the regulation of protein phosphatases is less well understood. In patients with heart failure, chronically elevated catecholamines upset the balance between β- and α₁-adrenergic receptors.15,16 Increased α₁-adrenergic signal-
ing in the myocardium of heart failure patients might contribute to defective cardiac conduction and to arrhythmias.

\( \alpha \)-Adrenergic signaling targets the transcription factor RTEF-1 (transcription enhancer factor-1–related factor) to upregulate gene expression in cultured cardiac myocytes. To better understand how RTEF-1 regulates cardiac gene expression and affects cardiac function in vivo, we made transgenic mice that overexpress RTEF-1 in the heart. Here, we show that RTEF-1 mice upregulate the expression of protein phosphatase 1 \( \beta \) (PP1\( \beta \)) in the heart, resulting in the dephosphorylation of cardiac connexins, impaired cardiac conduction, and arrhythmias.

**Methods**

**Experimental Animals**

RTEF-1 mice were generated on the FVB background using the rat \( \alpha \)-myosin heavy chain promoter to drive cardiac muscle–specific expression of the human RTEF-1 cDNA as described in the online-only Data Supplement. Nontransgenic littermates served as controls. Mice were maintained at the University of Pittsburgh barrier facility, and the Institutional Animal Care and Use Committee approved all animal procedures.

**Cardiac Myocyte Culture and Dye Transfer**

Neonatal ventricular myocytes were cultured in MEM supplemented with 5% BCS for 5 days, injected with Lucifer yellow and Texas Red dextran, and imaged as described in the online-only Data Supplement.

**Gel Shift Assay**

Gel shift was performed using an oligonucleotide containing the skeletal \( \alpha \)-actin promoter MCAT element, as described previously.

**Electrocardiography**

Surface ECGs were obtained as described previously from mice anesthetized with inhaled isoflurane. Heart rate, amplitude and duration of the QRS complex, and duration of the PR and QT intervals on the ECG were measured. Five consecutive QRS complexes were signal-averaged. QT interval was corrected for cycle length (QTc) using the formula of Mitchell et al.

**Radio telemetry**

Radio telemetry ECG monitors (Data Sciences) were implanted subcutaneously on the backs of mice anesthetized with 2.5% Avertin.

---

**Figure 1.** Generation of RTEF-1 transgenic lines and expression of RTEF-1 transgene. A, Diagram of rat \( \alpha \)-myosin heavy chain promoter driving human RTEF-1 transcript, including natural 5’ UTR, non-AUG leucine initiation codon (Leu, GCCCTTG, compared with Kozak consensus sequence GCCATGG), and natural 3’ UTR. B, Genomic Southern analysis shows that founder 1 mice integrated transgene in Y chromosome, unlike founders 2 and 3. End RTEF-1, endogenous RTEF-1 fragment; TG RTEF-1, human RTEF-1 transgene. C, Northern blot analysis of heart RNA reveals that only founder 2 and 3 mice express transgene (arrows). D, Western blots of cardiac protein extracts from wild-type (WT), RTEF-1 transgenic founder 2 (RTEF-1 F2), and founder 3 (RTEF-1 F3) mice reveal presence of human RTEF-1 protein (arrow). E, Gel shift assay shows elevated levels of high-mobility MCAT binding factor (RTEF-1, bracket) in founder 2 and 3 nuclear extracts. Open arrowhead, endogenous MCAT binding factor.
(IP), and telemetry ECGs were recorded from conscious mice and analyzed as described previously.19

Intracardiac Electrophysiology
Intracardiac electrophysiology studies were conducted as described previously.21 Baseline cardiac cycle intervals were measured, including the RR, PR, QRS, QT, AH, and HV intervals.

Optical Mapping Studies
Optical mapping of the cardiac action potential using the voltage-sensitive dye di-4-ANEPPS was performed on isolated perfused hearts as described previously22 and in the online-only Data Supplement. Atrial apparent velocities were measured during spontaneous depolarization in sinus rhythm, whereas ventricular velocities were measured during ventricular pacing (200-ms cycle length).

Western Blot Analysis
Western blots were probed with antibodies to human RTEF-1,18 connexin40, and connexin43 (Chemicon), a nonphosphorylated C-terminal peptide of connexin43 (Zymed), glyceraldehyde-3-phosphate dehydrogenase (G3PDH, Research Diagnostics), PKA, PKC, PKG, PP1, PP2A, and PP2B (Santa Cruz Biotechnology). Horseradish peroxidase–conjugated secondary antibodies were used to detect specific primary antibodies. Blots were developed by chemiluminescence, and bands were quantified by densitometry.

Northern Blot Analysis
RNA from whole hearts pooled from 2 male and 2 female littermate control mice and 2 male and 2 female RTEF-1 mice was size-fractionated, transferred to nylon membranes, and probed with radiolabeled cDNAs for various protein phosphatases obtained from IncyteGenomics. Signals were obtained on a phosphor storage screen (Molecular Dynamics) and normalized to G3PDH mRNA.

Confocal Microscopy
Frozen heart sections postfixed in 2% paraformaldehyde were incubated with primary antibodies, followed by appropriate secondary antibodies labeled with different fluoros. Sarcomeric actin was visualized by use of phallloidin conjugated with Alexa 647 (Molecular Probes). Sections were imaged on an Olympus Fluoview/Coherent Mira Multiphoton BX61 Microscope.

Data Analysis
Numerical data, reported as mean±SEM, were analyzed using the SPSS program by univariate multifactorial ANOVA or by Student’s t test and were considered significant at the P<0.05 level. Western blots were quantified by densitometry and correlated with ECG data.

Results
Transgenic mice with cardiac-specific expression of human RTEF-1 were made using a 2.2-kb fragment of the rat αMyHC promoter (Figure 1A). Genomic Southern blots determined that Founder 1 (Fndr 1) integrated 30 copies of the transgene into the Y chromosome, whereas founder 2 and 3 progeny had 2 and 20 copies of the transgene, respectively (Figure 1B). Northern blot analysis revealed RTEF-1 transgene expression only in founder 2 and 3 progeny (Figure 1C). Human RTEF-1 protein was detected in cardiac protein extracts by Western blot analysis using an antibody specific to human RTEF-1 (Figure 1D).19 Gel mobility shift assay using an oligonucleotide with a TEF-1 factor binding site (MCAT) showed that the human RTEF-1 protein is functional (Figure 1E). As expected, RTEF-1 mice showed upregulated expression of β-myosin heavy chain (data not shown), consistent with increased
expression of RTEF-1, a transcription factor targeted by α₁-adrenergic signaling.¹⁷,¹⁸

RTEF-1 mice from both lines have a cardiac conduction defect as early as 2 weeks after birth but survive well into adulthood with prolongation of the PR interval and QRS duration measured by surface ECGs (Figure 2A, Table). PR prolongation could result from a conduction delay in the atrial myocardium, at the AV node, and/or in the bundle of His, and prolonged QRS might indicate a delay in ventricular conduction. A significant prolongation of the AH interval but not the HV interval was detected by intracardiac electrophysiology, suggesting a conduction delay above the bundle of His (Figure 2B; control, AH = 21 ± 0.4 ms, n = 6; RTEF-1 transgenic, AH = 30 ± 2 ms, n = 6, P = 0.001). Optical mapping of action potentials in isolated perfused hearts demonstrated slower conduction over the surface of the atrial and ventricular myocardium (Figure 2C). Mean atrial conduction velocities were 49.6 ± 1.4 cm/s (n = 4) in control mice and 40.4 ± 6.0 cm/s in RTEF-1 mice (n = 5, P < 0.05). Mean ventricular velocities were 53.1 ± 6.1 cm/s (n = 5) in control mice and 38.3 ± 9.5 cm/s in RTEF-1 mice (mean age, 4.5 months, n = 7, P < 0.02). The early onset of PR and QRS prolongation indicates that the conduction defects are intrinsic and not secondary to atrial dilatation and remodeling (see Figure 4).

Accompanying the conduction defect, spontaneous transient episodes of supraventricular tachyarrhythmias were detected by ECG telemetry in 4 of 4 conscious founder 3 RTEF-1 mice 4 to 6 months old but not in controls (Figure 3, A and B). No ventricular arrhythmias were detected by ECG telemetry. By 12 months, nearly all the founder 3 RTEF-1 mice tested by surface ECG (7 of 8) appeared to

| ECG Parameters From Littermate Control and RTEF-1 Mice |
|----------------|--------|----------|---------|----------|
| Age/Mouse      | PR (ms) | QRS (ms) | QTc (ms) | QRS Amplitude (mV) |
| 2 Weeks        |        |          |         |                      |
| Control (n=12) | 33 ± 0.8 | 10 ± 0.3 | 42.9 ± 1.9 | 0.19 ± 0.01 |
| TG (n=5)       | 38 ± 1.9 | 16 ± 0.6 | 49.1 ± 0.9 | 0.23 ± 0.02 |
| 2 Months       |        |          |         |                      |
| Control (n=4)  | 28 ± 0.5 | 20 ± 0.9 | 48 ± 1.5 | 2.72 ± 0.37 |
| TG (n=4)       | 38 ± 1.2 | 24 ± 0.8 | 46 ± 1.5 | 3.76 ± 0.59 |
| 4 Months       |        |          |         |                      |
| Control (n=5)  | 31 ± 1.4 | 18 ± 0.9 | 50 ± 3.6 | 2.14 ± 0.22 |
| TG (n=7)       | 48 ± 3.6 | 24 ± 0.8 | 47 ± 2.3 | 2.57 ± 0.38 |

**ANOVA**

<table>
<thead>
<tr>
<th>Factor</th>
<th>P (TG)</th>
<th>P (Age)</th>
<th>P (TG x Age)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (8)</td>
<td>32 ± 0.4</td>
<td>17 ± 0.1</td>
<td>54 ± 0.4 N/A</td>
</tr>
<tr>
<td>n (14)</td>
<td>42 ± 0.5</td>
<td>18 ± 0.1</td>
<td>51 ± 0.3 N/A</td>
</tr>
<tr>
<td>P</td>
<td>P &lt; 0.01</td>
<td>P = 0.00</td>
<td>P = 0.16</td>
</tr>
<tr>
<td>12 Months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (4)</td>
<td>32 ± 1</td>
<td>22 ± 0.5</td>
<td>48 ± 1.5 2.28 ± 0.3</td>
</tr>
<tr>
<td>n (8)</td>
<td>N/A†</td>
<td>26 ± 1.1</td>
<td>61 ± 3.2 1.94 ± 0.2</td>
</tr>
<tr>
<td>P</td>
<td>P = 0.015</td>
<td>P &lt; 0.0001</td>
<td>P = 0.33</td>
</tr>
</tbody>
</table>

ANOVA was performed for ECG data from founder 3 RTEF-1 mice anesthetized with isoflurane at ages 2 weeks to 4 months to test for the effect of transgene (TG) and age and their interaction on the PR interval, QRS duration, QTc interval, and QRS amplitude.

†Seven of 8 founder 3 RTEF-1 mice at 12 months were not in sinus rhythm. Student t-test compared means at 6.5 and 12 months. All significant values are in boldface type.

**Figure 3.** Atrial arrhythmias in RTEF-1 mice. Telemetry ECG detected no arrhythmias in littermate control (A, n=4), whereas spontaneous episodes of tachyarrhythmias (SVT) were seen in founder 3 RTEF-1 mice (B, n=4). Isoproterenol further accelerated arrhythmias. One second of ECG telemetry is shown. C, Burst pacing from an intracardiac catheter–induced atrial flutter in a founder 2 RTEF-1 mouse. Upper tracing is surface ECG (lead 1), and lower tracing is intracardiac signal (IC). Ventricular depolarizations detected in surface ECG signal (QRS) correspond to high-amplitude spikes in IC signal. Oscillating lower-amplitude IC spikes are indicative of atrial flutter. Note that no P wave is detected by surface ECG during this episode. When atrial arrhythmia converted spontaneously to sinus rhythm after 4 seconds, single low-amplitude spikes (A) corresponded to P wave in surface ECG.
be in sustained atrial fibrillation. In founder 2 RTEF-1 mice 6.5 months old but not in controls, burst pacing from an intracardiac catheter electrode produced transient episodes of atrial flutter/fibrillation (Figure 3C). Consistent with gene dosage and the onset of atrial arrhythmias, RTEF-1 mice develop atrial dilatation (Figure 4A) without atrial or ventricular myocardial hypertrophy (Figure 4B). Atrial dilatation was apparent at 4 months in founder 3 RTEF-1 mice, and by 10 months, many showed severe thrombosis (Figure 4, A and C) and died suddenly. Atrial enlargement developed more slowly in founder 2 mice (Figure 4A), and sudden death was less frequent than in founder 3 mice. No evidence of ventricular interstitial fibrosis was detected either by Masson’s trichrome staining for collagen in tissue sections at 1 month of age (Figure 4D, n=4) or by total collagen assessed by the hydroxyproline method (n=4, data not shown).

We examined changes in the expression of cardiac connexins that might account for the impaired conduction in RTEF-1 mice using Western blots (Figure 5). The connexin40 antibody recognized a 41-kDa and a 40-kDa form of connexin40 (Figure 5, A and B) and does not cross-react with connexin43 (data not shown). The 40-kDa band corresponds to the dephosphorylated protein, because treatment of protein extracts with phosphatase increases the 40-kDa band (Figure 5A). In the atria of RTEF-1 mice, connexin40 was dephosphorylated, and this was associated with prolonged PR intervals (n=5, Figure 5B) and could account for slower atrial conduction observed by optical mapping (Figure 2C). In parallel, connexin43 dephosphorylation in the ventricular myocardium was also correlated with QRS prolongation (Figure 5C, r=0.703, P<0.05) and could account for slower ventricular conduction observed by optical mapping (Figure 2C). Connexin43 levels were not decreased in RTEF-1 mice (Figures 5C and 6).

Connexin40 and connexin43 dephosphorylation might occur through loss of a protein kinase or upregulation of a protein phosphatase. No significant change was seen in PKA, PP1α, PP1γ, PP2Ac, or PP2Bα (calcineurin Aα) at the protein level and/or at the mRNA level in the hearts of RTEF-1 mice. In contrast, PP1β levels were elevated and correlated with dephosphorylated Cx43 (Figure 5, D and E, r=0.722, P<0.05) and with QRS duration (r=0.903, P<0.005). PP1β overexpressed in HeLa cells transiently transfected with connexin40 expression vector dephosphorylated connexin40 in HeLa cells (Figure 5F).

Confocal microscopy revealed that dephosphorylated connexin43 is colocalized with total connexin43 at the gap junctions (Figure 6), demonstrating that gap-junction as-

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Progressive atrial dilatation in RTEF-1 mice. A, Atrial weights are significantly elevated in Founder 3 RTEF-1 mice compared with littermate controls and become significantly elevated only in old Founder 2 mice. Elevated atrial weights reflect distension of atrial myocardium and presence of organized thrombus. All values are mean±SEM, n=5. *P<0.05. Mean ages are indicated in weeks. B, Ventricular weights are not significantly different between age-matched founder 2 and founder 3 RTEF-1 mice and littermate controls. C, At 10 months, atrial dilatation often associated with thrombus was prevalent in founder 3 but less so in founder 2 RTEF-1 mice. D, At 1 month, size of atrium in a male founder 3 RTEF-1 mouse appears normal in a coronal section through tricuspid valve. Masson’s trichrome reveals collagen (blue) in valves but not in atrial or ventricular myocardium.
Assembly is not impaired in RTEF-1 mice. A marked increase in the level of dephosphorylated connexin43 was seen in RTEF-1 hearts, unlike littermate control mice, in which most of the connexin43 is phosphorylated, consistent with our Western blot analysis (Figure 5C).

To measure intercellular coupling, the fluorescent dye Lucifer yellow was injected into cultured neonatal RTEF-1 and control ventricular myocytes (Figure 7). Dye transfer was markedly reduced between cardiac myocytes of RTEF-1 mice (Figure 7E) compared with controls (Figure 7B). Dye spread less than 1 tier between RTEF-1 myocytes (0.71 tier ±0.19, n=17 injections) but up to 2 tiers of control myocytes (2.00 tiers ±0.22, n=27 injections, P<0.001) from the injected myocyte identified by coinjection of Texas Red dextran (Figure 7, A and D). RTEF-1 transgene expression was confirmed by Western blot analysis (Figure 7G).

**Discussion**

Many transgenic mouse models show abnormal conduction with PR prolongation and/or high-degree heart block. Decreased connexin abundance accounts for the conduction defect in some but not all transgenic models. Slow conduction in the ventricle could lead to reentrant arrhythmias and sudden death. Here, we report the first in vivo model in which chronic dephosphorylation of cardiac connexins, without loss of expression, is associated with defective cardiac conduction.

Conductance of connexin40 gap junctions in cultured cells is increased on phosphorylation of human connexin40 by PKA. Consistent with phosphorylation controlling gap-junction conductance, dephosphorylation of connexin40 is associated with defective atrial conduction in RTEF-1 mice. As in connexin40-null mice, burst pacing induces atrial arrhythmias in RTEF-1 mice. With age, RTEF-1 mice develop spontaneous atrial arrhythmias that probably contribute to progressive atrial dilatation. The extent of PR prolongation is similar between RTEF-1 and the connexin40-null mutant mice, suggesting that dephosphorylation produces a functional ablation of connexin40 and could account for the susceptibility of RTEF-1 mice to induced and spontaneous atrial arrhythmias. However, unlike the connexin40-null mouse, RTEF-1 transgenic mice do not show prolongation of the HV interval. This most likely reflects the absence of α-myosin heavy chain and RTEF-1 transgene expression in the bundle of His in the mouse.

Delayed conduction in the ventricle during myocardial ischemia is associated with transient dephosphorylation of connexin43. In RTEF-1 mice, chronic dephosphorylation of connexin43 is correlated with defective ventricular conduction (Figure 5C). Lucifer yellow dye injections confirmed that coupling through connexin43 gap junctions
is impaired between RTEF-1 ventricular myocytes. Mice with a conditional deletion of cardiac connexin43 after birth die suddenly and exhibit a 50% reduction in ventricular conduction velocity measured by optical mapping. As expected, this is more severe than the 30% to 40% reduction in ventricular conduction velocity seen in RTEF-1 mice.

Phosphorylation of cardiac connexin43 affects the rates of gap-junction assembly and turnover. Localization of C-terminal dephosphorylated connexin43 at the gap junction would suggest that gap-junctional assembly is not grossly affected in RTEF-1 mice. However, in vitro dye transfer experiments indicate that gap junctions between RTEF-1 cardiac myocytes are functionally uncoupled.

Recent evidence suggests that type 1 protein phosphatases (PP1s) dephosphorylate cardiac connexin43 and lower gap-junction conductance, although the isoform that mediates this effect is not known. PP1-selective inhibitory protein I-2 in isolated ventricular cardiac myocytes prevents the decline in gap-junction conductance that occurs in the absence of ATP infusion. Because protein kinases require ATP to maintain the correct phosphorylation status of cardiac connexins, inhibition of protein kinase activity and unopposed PP1 activity rapidly lower junctional conductance, presumably by dephosphorylating connexin43. Before heart failure, compensatory hypertrophy induced by myocardial infarction is associated with increased PP1 activity. Moreover, cardiac PP1 levels are upregulated in patients with end-stage heart failure. Interestingly, there is no significant ventricular hypertrophy in RTEF-1 mice, suggesting that the signals upstream of RTEF-1 that produce hypertrophy are not activated in these mice. Although RTEF-1 probably affects the expression of many downstream targets, our study shows that chronically increased PP1 levels in vivo are associated with impaired conduction. This provides evidence for the role of PP1 in the regulation of gap-junctional conductivity.
one mechanism by which heart failure may lead to conduction disease, arrhythmias, and sudden death.

In the RTEF-1 mouse, upregulation of PP1β mRNA levels suggests that activation occurs at the level of transcription. If so, this would be the first study to link a transcription factor to the regulation of protein phosphatase gene expression. It will be interesting to characterize the PP1β promoter to understand how this gene is regulated in cardiac myocytes. It remains uncertain whether the activation of protein phosphatases and the dephosphorylation of cardiac connexins underlie some forms of human conduction disease and contribute to arrhythmias in heart failure. If so, this RTEF-1 transgenic model may be useful to develop pharmacological interventions that can selectively block PP1β, restore phosphorylation of connexins, and improve cardiac conduction.

Acknowledgments

Dr Stewart was supported by a Grant-in-Aid from the American Heart Association (AHA), by National Institutes of Health (NIH) grant HL-57211, and by an award from the Competitive Medical Research Fund of The University of Pittsburgh Medical Center Health System. Dr Salama was supported by NIH grant HL-59614, and B. London by NIH grants HL-58030 and HL-66096. Dr Baty is a recipient of a Burroughs Wellcome Hitchings-Elion Fellowship. Dr Baker was supported by a predoctoral fellowship, and T. Ueyama by a postdoctoral fellowship of the AHA.

References

Transcription Enhancer Factor-1-Related Factor-Transgenic Mice Develop Cardiac Conduction Defects Associated With Altered Connexin Phosphorylation


_Circulation_. 2004;110:2980-2987; originally published online November 1, 2004; doi: 10.1161/01.CIR.0000146902.84099.26

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/110/19/2980

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2004/11/08/01.CIR.0000146902.84099.26.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/