UltraRapid Communication

Enhanced Na\(^+\) Channel Intermediate Inactivation in Brugada Syndrome

Dao W. Wang, Naomasa Makita, Akira Kitabatake, Jeffrey R. Balser, Alfred L. George, Jr

Abstract—Brugada syndrome is an inherited cardiac disease that causes sudden death related to idiopathic ventricular fibrillation in a structurally normal heart. The disease is characterized by ST-segment elevation in the right precordial ECG leads and is frequently accompanied by an apparent right bundle-branch block. The biophysical properties of the SCN5A mutation T1620M associated with Brugada syndrome were examined for defects in intermediate inactivation (I\(_{\text{im}}\)), a gating process in Na\(^+\) channels with kinetic features intermediate between fast and slow inactivation. Cultured mammalian cells expressing T1620M Na\(^+\) channels in the presence of the human \(\beta_1\) subunit exhibit enhanced intermediate inactivation at both 22°C and 32°C compared with wild-type recombinant human heart Na\(^+\) channels (WT-hH1). Our findings support the hypothesis that Brugada syndrome is caused, in part, by functionally reduced Na\(^+\) current in the myocardium due to an increased proportion of Na\(^+\) channels that enter the I\(_{\text{M}}\) state. This phenomenon may contribute significantly to arrhythmogenesis in patients with Brugada syndrome. The full text of this article is available at http://www.circresaha.org. (Circ Res. 2000;87:e37-e43.)

Key Words: Brugada syndrome ■ Na\(^+\) channel ■ SCN5A ■ slow inactivation

Sudden cardiac death due to ventricular arrhythmias continues to be a common cause of death in industrialized nations. Recent advances have elucidated the molecular genetic basis of several forms of the congenital long-QT syndrome and the more recently defined Brugada syndrome. Disease-producing mutations have been identified in several cardiac ion channel genes including SCN5A, which encodes the voltage-gated Na\(^+\) channel \(\alpha\) subunit.1,2 Understanding the pathogenesis of these uncommon familial syndromes will provide a foundation of knowledge that will help unravel the complex pathophysiology of more common cardiac arrhythmia syndromes.

Brugada syndrome is a form of idiopathic ventricular fibrillation that exhibits a characteristic ECG pattern consisting of ST elevation in the right precordial leads and an apparent right bundle-branch block.3 Inheritance appears to be autosomal dominant, and there is often a family history of unexplained sudden death. On mechanistic grounds, several cardiac ion channels were identified as potential candidate genes for Brugada syndrome. In 1998, Chen et al2 reported the discovery of mutations in SCN5A in families with Brugada syndrome. Subsequently, additional SCN5A mutations have been identified, and many laboratories have contributed to the functional characterization of these alleles using heterologously expressed recombinant Na\(^+\) channels.4–7

A prevailing concept regarding the pathophysiology of Brugada syndrome is that heterogeneity of repolarization exists across the right ventricular wall, in part due to asymmetric transmural expression of the transient outward current, I\(_{\text{to}}\).8 Theoretical arguments and experimental data support the notion that a reduction in Na\(^+\) current will further amplify the transmural voltage gradient during repolarization and greatly increase the propensity for reentrant arrhythmia.9 Some SCN5A mutations associated with Brugada syndrome, especially mutations associated with aberrant exon splicing or a frameshift, are quite consistent with this pathophysiological concept. In contrast, some missense mutations in SCN5A yield functional channels with subtle biophysical defects.

Studies of recombinant, heterologously expressed Na\(^+\) channels have been valuable for elucidating biophysical defects in mutant Na\(^+\) channels that may be mechanistically compatible with the Brugada phenotype. The most well-studied missense mutation is a substitution of threonine 1620 by methionine (T1620M). In the initial report of this mutation, Chen et al2 demonstrated subtle changes in the voltage dependence of steady-state inactivation and enhanced recovery from fast inactivation when the recombinant Na\(^+\) channel mutant was expressed in Xenopus oocytes. These gating defects were surprisingly inconsistent with a loss of function phenotype.10,11 More recently, Dumaine et al12 have emphasized the importance of conducting electrophysiological re-
cordings at more physiological temperatures using cultured mammalian cells to define the precise biophysical defect in this mutant allele. This study demonstrated that at near physiological temperatures, T1620M Na1 channels exhibit more rapid fast inactivation kinetics than wild-type Na1 channels. Although this biophysical defect would explain diminished Na1 channel current density in Brugada syndrome, these studies were carried out in the absence of the β1 subunit, which our studies suggest may be critical for full expression of the T1620M gating defect.11 Despite the apparent extensive analysis of this single missense mutation in Brugada syndrome, a clear, unifying pathophysiological mechanism related to specific biophysical defects associated with this disease has not emerged.

Recently, Veldkamp et al13 described the functional disturbances exhibited by an unusual SCN5A insertion mutation (1795insD) associated with both clinical phenotypes of congenital long-QT syndrome and Brugada syndrome. The question remains whether other Brugada syndrome myocardium exhibit intermediate inactivation properties. By contrast at 32°C, current amplitude is less stable and may run down within 5 to 10 minutes after rupturing the cell membrane. By contrast at 32°C, current amplitude is less stable and may run down within 5 to 10 minutes after rupturing the cell membrane.

Materials and Methods

Heterologous Expression of Na1 Channels in tsA201 Cells

Cells (tsA201) were transiently transfected with pRcCMV-hH1 or pRcCMV-T1620M using SuperFect (Qiagen Inc) in combination with a bicistronic plasmid (pGFP-ires-hβ1) encoding green fluorescent protein (GFP) and the human β1 subunit (hβ1). Transfections used 1 μg of channel-encoding plasmid DNA and 1 μg of pGFP-IRES-hβ1. In some experiments, pGFP-IRES without hβ1 was cotransfected with pRcCMV-hH1 or pRcCMV-T1620M. Cells expressing GFP were selected for patch-clamp recording experiments. Transiently transfected cells were transferred to a heating chamber (Warner Instrument Corp) for electrophysiological measurements 40 to 72 hours after transient transfection.

Electrophysiology

Na1 currents were recorded using the whole-cell patch-clamp technique as described previously.17,18 Electrode resistance ranged from 0.8 to 1.5 MΩ. Data acquisition was carried out using an Axopatch 200B patch-clamp amplifier and pCLAMP v8.0 software (Axon Instruments, Inc). Currents were filtered at 5 kHz (~3 dB, four-pole Bessel filter) and digitized using an analog-to-digital interface (Digidata 1200B, Axon Instruments). Capacitance and series resistance were adjusted (70% to 85%) to obtain minimal contribution of the capacitive transients. The holding potential was −120 or −90 mV for all experiments, and details of each pulse protocol are given schematically in the figures and explained in the Results section.

The bath solution contained (in mmol/L) NaCl 145, KCl 4, CaCl2 1.8, MgCl2 1, HEPES 10, and glucose 10, pH 7.35 (adjusted with NaOH). The pipette solution (intracellular solution) contained (in mmol/L) NaF 10, CsF 110, CsCl 20, EGTA 10, and HEPES 10, pH 7.35 (adjusted with CsOH). In some experiments at 32°C, the following intracellular solution was used (in mmol/L): NaCl 35, CsF 105, EGTA 10, and HEPES 10, pH 7.35 (adjusted with CsOH). Osmolarity was adjusted to 310 mOsm with sucrose for all solutions.

Data Analysis

All data were analyzed by using pCLAMP v8.0 (Axon Instruments, Inc), and the figures were prepared using SigmaPlot v2000 (SPSS Inc). The time course of inactivation of macroscopic current was fit with a two-exponential function: I(t)/I∞ = A1 × exp(−t/τ1) + A2 × exp(−t/τ2). Steady-state availability was fit with the Boltzmann equation, I(t)/I∞ = [1 + exp(V − V1/2/kT)]−1 to determine the membrane potential for half-maximal activation (V1/2) and the slope factor k. Recovery from inactivation was analyzed by fitting data to two exponentials: I(t)/I∞ = A1 × [1 − exp(−t/τ1)] + A2 × [1 − exp(−t/τ2)]. All data were fit using a nonlinear least-squares minimization method.

Results

Characterization of T1620M Fast Inactivation

Initially, we examined the kinetics and steady-state voltage dependence of fast inactivation in cultured mammalian cells (tsA201) expressing either wild-type recombinant human heart Na1 channels (WT-hH1) or T1620M channels at near physiological temperature (32°C) in the absence and presence of hβ1. To facilitate voltage control at 32°C, we transfected cells with a minimal amount of plasmid DNA to achieve low-amplitude Na1 currents, used lower-resistance patch pipettes (0.8 to 1.0 MΩ), and tested cells of smaller size that tend to exhibit smaller currents and smaller capacitance transients. Data were collected only from cells that had peak current amplitudes of 1 to 3 nA. Current rundown was not observed at room temperature where Na1 currents stabilize within 5 to 10 minutes after rupturing the cell membrane. By contrast at 32°C, current amplitude is less stable and may run down with variable rate and extent. We carefully monitored time-dependent changes in peak current amplitudes and selected data for statistical analysis from cells exhibiting <5% rundown over the time course of the experiment. To minimize the contribution of time-dependent shifts of channel availability,18 all data were collected within 20 minutes after establishing the whole-cell patch clamp.

Characterization of T1620M Fast Inactivation

Initially, we examined the kinetics and steady-state voltage dependence of fast inactivation in cultured mammalian cells (tsA201) expressing either wild-type recombinant human heart Na1 channels (WT-hH1) or T1620M channels at near physiological temperature (32°C) in the absence and presence of hβ1. To facilitate voltage control at 32°C, we transfected cells with a minimal amount of plasmid DNA to achieve low-amplitude Na1 currents, used lower-resistance patch pipettes (0.8 to 1.0 MΩ), and tested cells of smaller size that tend to exhibit smaller currents and smaller capacitance transients. Data were collected only from cells that had peak current amplitudes of 1 to 3 nA. Current rundown was not observed at room temperature where Na1 currents stabilize within 5 to 10 minutes after rupturing the cell membrane. By contrast at 32°C, current amplitude is less stable and may run down with variable rate and extent. We carefully monitored time-dependent changes in peak current amplitudes and selected data for statistical analysis from cells exhibiting <5% rundown over the time course of the experiment. To minimize the contribution of time-dependent shifts of channel availability,18 all data were collected within 20 minutes after establishing the whole-cell patch clamp.
channels exhibit similar kinetics and voltage dependence of recovery from fast inactivation. Most channels (>95%) recovered rapidly with time constants <2 ms, and there were no significant differences between WT-hH1 and T1620M. To examine the voltage-dependent loss of channel availability due to fast inactivation, we used a 10-ms prepulse of varying potentials from −160 to −10 mV followed by a −10-mV test pulse. In the presence of hβ4, cells expressing T1620M exhibit a midpoint (V_{1/2}) of channel availability that is shifted 6 mV toward more positive potentials compared with WT-hH1 (WT-hH1+hβ4: −72.5±1.3 mV, n=9; T1620M+hβ4: −66.3±0.9 mV, n=13; P<0.01). This result is consistent with previously reported findings examining T1620M in *Xenopus* oocytes,2 or mammalian cells1,12 although Baroudi et al19 did not observe this shift in tsA201 cells using voltage-clamp protocols similar to those we used.

**Characterization of Intermediate Inactivation (I_M)**

Na⁺ channels exhibit multiple types of inactivation that can be distinguished by their time course. Fast inactivation occurs over a few milliseconds and is the classical type of inactivation gating described originally by Hodgkin and Huxley.20

---

Figure 1. Whole-cell current recordings of WT-hH1 and T1620M at 32°C. Na⁺ channels were expressed by transient transfection in tsA201 cells in the absence (A and B) or presence (C and D) of hβ4, and currents were recorded at various membrane potentials from −80 to +50 mV in 10-mV increments from a holding potential of −120 mV. E, Voltage dependence of fast-inactivation time constants for WT-hH1 (open symbols) and T1620M (filled symbols). Triangles indicate τ1 values, and circles indicate values for τ2. F, Voltage dependence of fast-inactivation time constants for WT-hH1+hβ4 (open symbols) and T1620M+hβ4 (filled symbols). Triangles indicate τ1 values, and circles indicate values for τ2. Results are presented as mean±SEM for 6 to 15 cells.

Figure 2. Time course of recovery from fast inactivation and voltage dependence of Na⁺ channel availability of WT-hH1+hβ4 and T1620M+hβ4 at 32°C. A, Time course of recovery from fast inactivation studied using the two-pulse protocol shown in the inset. Time constants and fractional amplitudes (given in parentheses) are as follows: WT-hH1+hβ4, τ1=1.6±0.3 ms (95.2±7.5%), τ2=19.3±10.7 ms (4.8±7.5%), n=9; T1620M+hβ4, τ1=1.9±0.7 ms (87.9±10.6%), τ2=43.8±15.8 ms (12.1±10.6%), n=8 (comparisons of time constants and fractional amplitudes between groups were not statistically significant). B, Na⁺ channel availability for WT-hH1+hβ4 (n=9) and T1620M+hβ4 (n=13) recorded using the pulse protocol shown in the inset and fitted with Boltzmann distributions (solid lines). Values for half-maximal voltage (V_{1/2}) are provided in the text. Results are presented as mean±SEM.
Slow inactivation is a distinct gating process that occurs over a time period of a few hundred milliseconds to tens of seconds and is important physiologically for determining the number of channels available for firing action potentials.\(^1\) One form of slow inactivation that has drawn attention recently has intermediate kinetics and has been designated as \(I_{St}\).\(^15\) Because during cardiac action potentials the myocyte membrane is depolarized for periods sufficient to elicit \(I_{Na}\), we examined the impact of the T1620M mutation on \(I_{St}\) to address the question whether this could be a factor in the pathogenesis of Brugada syndrome.

Initial experiments were designed to characterize the development of \(I_{Na}\) at room temperature (22°C) using a two-pulse protocol illustrated in Figure 3A (inset) that includes an initial conditioning prepulse to −10 mV (P1), followed by a 20-ms step to −120 mV to remove fast inactivation, and a final test pulse (P2) to −10 mV to assess channel availability. With progressively longer prepulse durations, \(Na^+\) current diminished more in cells coexpressing T1620M and hβ1, than those transfected with WT-hH1 and hβ1. This effect became marked as prepulse durations exceeded 200 ms, consistent with \(I_{St}\) kinetics.\(^13,15\) At a prepulse duration of 1000 ms, 5.4±1% of WT-hH1 current was inactivated compared with 16.3±2% for T1620M (P<0.01). Interestingly, no difference in the development of inactivation with this pulse protocol was observed between T1620M and WT-hH1 in the absence of hβ1 (for P1=1000 ms, 27±1% WT-hH1 current was inactivated, n=6, versus 29±3% for T1620M, n=7).

Additional experiments were performed to characterize differences in recovery and steady-state voltage dependence of \(I_{St}\) between WT-hH1 and T1620M. Figure 4A illustrates differences in the kinetics of recovery from inactivation induced by a 1000-ms depolarization to −10 mV for WT-hH1 and T1620M, both coexpressed with hβ1. Analysis of these data revealed two kinetic components reflected in the time constants \(\tau_r\) (WT-hH1+hβ1; 3.5±0.4 ms, n=11; T1620M+hβ1; 6.3±0.7 ms, n=13, P<0.05) and \(\tau_m\) (WT-hH1+hβ1; 73.1±10.3 ms; T1620M+hβ1; 88.1±9.5 ms; NS). Notably, the time constant that best reflects recovery from intermediate inactivation (\(\tau_m\)) is not changed by the mutation. The faster component of recovery from inactivation, reflected in the \(\tau_r\) value, predominates in both channels and is slower in the mutant (\(\tau_r\) probably represents a mixture of fast and intermediate inactivation in this experiment). More importantly, there is a significant difference in the partitioning between the two kinetic components exhibited by T1620M such that a much greater...
propportion of mutant channels recovers with the slower time constant (WT-hH1+β1: τ₁ amplitude = 7.4±0.9%, versus T1620M+β1: τ₁ amplitude = 26.1±2.5%, P<0.001). We interpret this as evidence that T1620M channels more readily enter the intermediate inactivated state during the 1000-ms conditioning pulse than WT-hH1. Once inactivated, both WT-hH1 and T1620M channels recover from the intermediate state at similar rates.

Figure 4B illustrates the steady-state partitioning of WT-hH1 and T1620M channels into inactivated states over a wide range of membrane potentials. After a 1000-ms depolarization to induce intermediate inactivation, cells were briefly hyperpolarized (20 ms) to allow recovery from fast inactivation before channel availability was measured (see Figure 4B inset). In the presence of β1, the availability of mutant channels over a wide range of depolarized voltages was substantially lower than WT-hH1, consistent with enhanced partitioning of channels into the intermediate inactivated state.

Intermediate Inactivation at 32°C

The data presented above are consistent with enhanced entry of T1620M channels coexpressed with β1 into the intermediate inactivated state. The duration of the human cardiac action potential provides an appropriate time frame during which a significant degree of intermediate inactivation could develop. Therefore, the significant differences observed between WT-hH1 and T1620M could result in lower steady-state Na⁺ channel availability and reduced depolarizing myocardial Na⁺ current in the mutant, consistent with prevailing concepts concerning the pathophysiology of Brugada syndrome. To test whether this observation could be relevant to the in vivo situation, we repeated the experiment illustrated in Figure 3B at near physiological temperature (32°C). Figure 5 demonstrates a similar enhancement of Iₙ at this warmer temperature in cells expressing T1620M with β1. After a 1000-ms depolarization to −10 mV and a brief repolarizing step to −120 mV to allow recovery from fast inactivation, the proportional decrement in channel availability was ~2-fold greater for T1620M than WT-hH1 (WT-hH1+β1: 14.1±2.2%, n=5; versus T1620M+β1: 27.7±2.8%, n=13, P<0.01). To illustrate the potential physiological significance of this effect, we recorded peak Na⁺ currents at 32°C during a series of 500-ms test depolarizations to +10 mV at a rate simulating a cardiac cycle length of 0.52 seconds (115 bpm). Data shown in Figure 6 demonstrate that T1620M channels exhibit a significantly greater degree of activity-dependent loss of availability. Overall, our results support the conclusion that the Brugada syndrome Na⁺ channel mutation T1620M exhibits enhanced development of intermediate inactivation, and this biophysical defect could be a significant factor in the pathogenesis of this disease.

Discussion

Brugada syndrome is a rare but highly informative condition of susceptibility to potentially lethal ventricular tachyarrhythmias that provides an important model for understanding the pathomechanism underlying more common arrhythmia syndromes.22,23 Perhaps the most attractive and well-substantiated hypothesis to explain the cellular basis of Brugada syndrome involves reduced myocardial Na⁺ current and the resultant imbalance of inward and outward currents particularly in the right ventricular epicardium where disproportionate expression of the transient outward current creates a transmural voltage gradient and dispersion of repolarization.8,24 This hypothesis has been validated by experimental animal models and by computational methods.9,12 The theory helps to explain the characteristic ECG pattern observed in patients with Brugada syndrome, provides a basis for understanding the effects of Na⁺-channel blocking agents to aggravate this phenotype, and may illustrate mechanisms
underlying acquired ventricular arrhythmia syndromes such as acute myocardial infarction.

Previous work to characterize the molecular genetics and molecular physiology of Brugada syndrome has provided evidence that reduced myocardial Na\(^+\) current results from disease-associated mutations in SCN5A. Two of the first reported Brugada syndrome mutations severely disrupt the coding potential of the SCN5A mRNA and are expected to result in nonfunctional Na\(^+\) channels.\(^1\) Other missense mutations in SCN5A have been described and a few have been characterized using recombinant Na\(^+\) channels expressed heterologously in cultured cells. At least one mutant allele (R1432G) is nonfunctional,\(^2\) but other mutations do not prevent expression of functional Na\(^+\) channels. The first identified missense mutation T1620M, which we have re-studied here, has been evaluated by several laboratories, and variable defects in fast gating properties have been described.\(^2,11,12,19\) These defects include shifts in the steady-state voltage dependence of fast inactivation and activation as well as changes in the rate of recovery from fast inactivation or entry into the fast inactivated state. These findings are in contrast to those characterizing prototypic LQT3 SCN5A mutations in which there is an almost universally observed defect in fast inactivation manifest as a small noninactivating current component during long depolarizations. The variable results reported by laboratories studying T1620M may be attributable to several factors. It is clear that subtle phenotypic differences in the character of fast inactivation may stem from the nature of the heterologous cells used (Xenopus oocytes versus culture cells),\(^19\) the presence or absence of the \(\beta\) subunit,\(^11\) temperature,\(^12\) and subtle differences in voltage-clamp protocols and recording solutions.

In the present study, we selected cultured mammalian cells as the heterologous model and performed experiments predominantly in the presence of h\(\beta\)\(_{1}\), which is known to be expressed in heart\(^23\) and likely interacts with the cardiac Na\(^+\) channel \(\alpha\) subunit.\(^26\) We also examined fast gating behavior of the mutant channel at near physiological temperature based on the report of Dumaine et al.\(^12\) Our results suggests that a small but significant shift in the voltage dependence of steady-state fast inactivation occurs in cells expressing T1620M, but we find less evidence for a substantial effect of the mutations on fast inactivation. There are two possible explanations for differences between our data and those reported by Dumaine et al.\(^12\) Our experiments used a lower intracellular Na\(^+\) concentration (10 versus 35 mmol/L), and our values for the time constants of fast inactivation for WT-hH1 are smaller than those reported in the other study.\(^12\) Unfortunately, it is not possible to precisely reconcile these differences between our study and previous work performed on T1620M. However, there is little doubt that defects in fast gating can theoretically contribute to the pathophysiology of reduced Na\(^+\) current associated with Brugada syndrome. As demonstrated by the work of Dumaine et al.,\(^12\) subtle changes in the kinetics of fast inactivation are predicted to exert a substantial effect on the morphology of epicardial action potentials as deduced from computer modeling experiments. These findings are clearly in line with the prevailing hypoth-

esis of Brugada syndrome and further validation of these results will be important.

In addition to defects in fast gating, we examined whether concomitant alterations in inactivation processes occurring with a slower time course might contribute to the biophysical dysfunction of T1620M channels. This work was motivated by the recent observations of Veldkamp et al\(^13\) describing an unusual SCN5A mutation associated with the clinical phenotypes of both long-QT and Brugada syndromes. This unusual mutation (1795insD) exhibits the sustained noninactivating current characteristic of prototypic LQT3 mutations as well as a previously undescribed enhancement of intermediate inactivation. This enhancement of intermediate inactivation is consistent with reduced steady-state Na\(^+\) current and is compatible with the Brugada syndrome hypothesis described above. Our experiments similarly demonstrate enhanced development of intermediate inactivation in the T1620M allele associated with the Brugada syndrome phenotype alone. Thus, our work demonstrates that an enhancement in a slow gating process may underlie the biophysical defect in Na\(^+\) channel mutations associated with Brugada syndrome.

Intermediate inactivation and other slow gating states exhibited by Na\(^+\) channels may be important determinants of the action of local anesthetic and antiarrhythmic agents in heart\(^15\) and may participate in the pathogenesis of inherited skeletal muscle Na\(^+\) channelopathies. In particular, mutations in the SCN4A Na\(^+\) channel associated with hyperkalemic periodic paralysis exhibit defects in both fast and slow gating.\(^27,28\) In this case, impairment of slow inactivation may contribute to the ability of depolarized muscle fibers to have sustained attacks of inexcitability and paralysis.\(^29\)

The structural basis for slow gating in Na\(^+\) channels may relate to several regions of the channel protein including the pore-forming segments. The T1620M allele is located in an extracellular loop between the S3 and S4 segments of domain 4 in the cardiac Na\(^+\) channel and therefore is not intimately associated with structures known to contribute to permeation. At this point, it is unclear whether this residue participates directly or indirectly in mediating intermediate inactivation or other slow gating processes. However, recent data indicate a role for the adjacent domain 4, S4 segment in slow inactivation.\(^30\)

In summary, we have described enhanced intermediate inactivation in a missense SCN5A mutation associated with the Brugada syndrome. This biophysical property of mutant Na\(^+\) channels may contribute to the overall pathogenesis of the disease by reducing steady-state myocardial Na\(^+\) current. These observations provide another perspective for understanding the molecular basis of ventricular arrhythmia syndromes and may help provide an additional functional target for drugs to treat these disorders.

Acknowledgments

This work was supported by grants from the National Institutes of Health (NS32387 to A.L.G., GM56307 to J.R.B.) and the American Heart Association. A.L.G. and J.R.B. are Established Investigators of the American Heart Association. We thank David Johns (Institute for Molecular Cardiology, Johns Hopkins University, Baltimore, Md) for providing the GFP-IRES vector.
References

Enhanced Na\(^+\) Channel Intermediate Inactivation in Brugada Syndrome
Dao W. Wang, Naomasa Makita, Akira Kitabatake, Jeffrey R. Balser and Alfred L. George, Jr

_Circ Res._ 2000;87:e37-e43
doi: 10.1161/01.RES.87.8.e37

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/87/8/e37

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ 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 Research_ is online at:
http://circres.ahajournals.org/subscriptions/