Electrical remodeling of cardiac myocytes from mice with heart failure due to the overexpression of tumor necrosis factor-α

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Inflammatory cytokines, including tumor necrosis factor-α (TNF-α), appear to play a significant role in the pathophysiology of heart failure (5, 15, 51). TNF-α has direct negative inotropic effects on Ca2+ handling in cardiac myocytes mediated in part by nitric oxide (16, 18, 42) and decreases myocardial contractility when infused into animals (7, 43). Inflammatory cytokines, cardiac vascular smooth muscle cells, and cardiac myocytes can all synthesize and release TNF-α in the heart (12, 21). In addition, advanced heart failure is associated with increased serum concentrations and cardiac tissue levels of TNF-α (12, 13, 21, 29, 33, 39, 50, 55).

Transgenic mice that overexpress TNF-α selectively in the heart (TNF mice) have been extensively studied as a model of congestive heart failure (9, 25, 31, 36, 52). Affected mice develop a heart failure phenotype characterized by a mild inflammatory infiltrate, atrial dilatation, ventricular hypertrophy and dilation, diminished β-adrenergic responses, decreased ventricular ejection fraction, and interstitial fibrosis. Moreover, the mice develop clinical congestive heart failure with lethargy, dyspnea, pleural effusions, arrhythmias, and premature death. Recently, we have used optical mapping with voltage- and calcium-sensitive dyes to show that hearts and myocytes isolated from TNF mice have prolonged action potential durations (APDs) and altered intracellular Ca2+ transients with decreased peak systolic Ca2+, elevated diastolic Ca2+, and slower kinetics (25, 36). These marked changes in cellular electrical properties suggest that TNF-α overexpression in the heart results in the remodeling of the ion channels that underlie the generation of the action potential. In a prior microarray study, RNA expression of several K+ channels was significantly decreased in the left ventricle of TNF mice (53). The direct effect of chronic TNF-α expression on ion channel protein expression and on ionic currents has not been studied, however.

The present study uses the whole cell voltage-clamp technique on isolated cardiac myocytes from TNF mice with heart failure and control littermates to determine whether changes in outward K+ and/or inward Ca2+ currents underlie the observed APD prolongation and abnormal Ca2+ handling. We show downregulation of several K+ currents that may affect APD and contribute to the increased vulnerability to arrhythmias.

MATERIALS AND METHODS

Generation of transgenic mice. All studies were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh. Heterozygous female TNF-α transgenic mice were bred...
with FVB male controls to generate female TNF mice and wild-type littermate controls. Experiments were performed using 5-mo-old female TNF mice. Female mice were chosen due to the high mortality of male TNF mice (28). Age- and sex-matched nontransgenic littermates or FVB controls were used for control K⁺ and Ca²⁺ current measurements.

**Isolation of ventricular myocytes.** Cardiac myocytes were isolated from the left ventricular free wall of Langendorff-perfused hearts. Briefly, mice were anesthetized with pentobarbital sodium (35 mg/kg ip) and injected with heparin (50 U ip), and the hearts were rapidly excised, cannulated, and perfused at 35–36°C with Tyrode solution containing (in mM) 126 NaCl, 4.4 KCl, 5 MgCl₂, 22 glucose, 5 Na pyruvate, 5 creatine, 20 taunine, 0.9 NaH₂PO₄, and 10 HEPES (pH adjusted to 7.35 with NaOH and gassed with 100% O₂). Each heart was perfused with a constant hydrostatic pressure resulting in a steady-state flow rate in the range of 2–3.5 ml/min for 3–5 min and then perfused with Tyrode solution plus 0.5 mg/ml collagenase (Worthington type 2, 319 U/mg), 0.02 mg/ml protease (Sigma, type XXIV), and 0.025 mM Ca²⁺. After a 50–100% increase of flow rate, indicative of adequate digestion, the heart was disconnected from the cannula and bathed in a low-Ca²⁺ Tyrode solution (0.025 mM Ca²⁺). The right ventricle and septum were removed, and single cardiac cells were obtained by gentle trituration of the tissue segments cut from the remaining portion of the left ventricle. Cell suspensions were filtered to remove undissociated heart fragments and collected by sedimentation. Isolated myocytes were resuspended in Tyrode solution containing 0.1 mM Ca²⁺ with 1 mg/ml BSA and stored for up to 10 h at room temperature for electrophysiological recordings.

**Voltage-clamp studies.** Membrane currents were recorded in the whole cell configuration of the voltage-clamp technique (22). To measure K⁺ currents, the pipette solution contained (in mM) 135 KCl, 1 MgCl₂, 10 EGTA, 10 HEPES, 5 glucose, 3 Mg₂⁺ATP, pH adjusted to 7.2 with KOH, and the external solution contained (in mM) 136 NaCl, 4 KCl, 2 CaCl₂, 2 MgCl₂, 10 HEPES, and 10 glucose, pH adjusted to 7.35 with NaOH. CoCl₂ (5 mM) and tetrodotoxin (TTX, 20 µM) were added to the external solution to block Ca²⁺ and Na⁺ currents, respectively.

Voltage-activated K⁺ currents that drive repolarization of the action potential in mouse ventricular myocytes are composed of several dominant components (64). These include 1) a transient outward K⁺ current (Iₒ) that consists of a fast component (Iₒfast) encoded by Kv4.2 and Kv4.3 (3) and a slow component (Iᵦslow) encoded by Kv1.4 and expressed mainly in myocytes from the septum (20); 2) a highly 4-aminopyridine (4-AP) sensitive rapidly activating, slowly inactivating K⁺ current (I_k,slow1) encoded by Kv1.5 (37); 3) a tetraethylammonium (TEA)-sensitive rapidly activating, slowly inactivating K⁺ current (I_k,slow2) encoded by Kv2.1 (63); and 4) a nonactivating sustained current (Iₛ).

K⁺ currents were activated by 4.5-s depolarizing voltage steps to potentials between −40 and +50 mV in 10-mV increments at 15-s intervals from a holding potential of −90 mV. Each voltage step was preceded by a prepulse of 20 ms to −20 mV to inactivate the inward Na⁺ current. In addition, TTX (20 µM) was added in the bathing solution (Fig. 1A). Total currents were initially fit to a double-exponential function I(t) = Aₒ + Afast,texp(−t/τfast) + Aslow,texp(−t/τslow), where Aₒ represents the amplitude of the steady-state current Iₒ, Afast represents the amplitude of Iₒ, and Aslow represents the amplitude of I_k,slow (64). We then used a scheme to separate the distinct K⁺ currents in control and TNF mice on the basis of the pharmacological responses, activation and inactivation kinetics, and activation threshold. 4-AP (50 µM) was added to selectively block a significant fraction of I_k,slow (Fig. 1B), and I_k,slow1 was determined by subtracting the currents in the presence of 50 µM 4-AP from the currents without the inhibitor (Fig. 1C). A second prepulse (+40 mV, 100-ms duration) was then applied to inactivate Iₒ (Fig. 1D), and Iₒ was measured by subtracting the currents before and after that prepulse (Fig. 1E). I_k,slow2 and Iₛ amplitudes were then determined by fitting the remaining K⁺ current traces obtained after the two prepulses (Fig. 1D) to a single-exponential function I(t) = Aₒ + Afast,texp(−t/τfast), where Aₒ and Afast represent the amplitudes of Iₒ and I_k,slow, respectively, and τfast = γAₒ represented inactivation of I_k,slow. All current amplitudes were normalized to the cell capacitances and expressed as densities (in pA/pF).

To measure Ca²⁺ currents the pipette solution contained (in mM) 140 CsCl, 4 MgCl₂, 10 HEPES, 10 EGTA, 4 Na₂ATP, pH adjusted to 7.3 with CsOH, and the external solution contained (in mM) 137 NaCl, 5.4 CsCl, 1.25 MgCl₂, 10 HEPES, 10 glucose, 1.5 CaCl₂, pH adjusted to 7.4 with NaOH. Voltage-gated L-type Ca²⁺ channel currents (I_Ca,L) were evoked by depolarizing voltage steps (250 ms in duration) to potentials between −40 and +60 mV in 10-mV increments at 5-s intervals from a holding potential of −85 mV. To isolate I_Ca,L from contaminating sodium currents (I_Na, TTX (10 µM) was added to the bath solution, and a 50-ms prepulse to −45 mV was used. To prevent contamination from K⁺ currents, K⁺ in the pipette and external solutions was replaced with Cs⁺. I_Ca,L was determined from the difference in the amplitude of the peak inward current and that at the end of the 250-ms pulse Ca²⁺ currents, filtered at 10 kHz before digitization, and analyzed offline using PulseFit (v8. HEKA, Lambrecht, Germany) with the Simplex optimization algorithm for curve fits.

**Reagents.** All reagents and chemicals were obtained from Sigma (St. Louis, MO) or Fischer Scientific (Fair Lawn, NJ). Collagenase type 2 was purchased from Worthington Biochemical (Lakewood, NJ) and TTX from Alomone Labs (Jerusalem, Israel).

**Western blot analysis.** Hearts from TNF mice and age-/gender-matched FVB control mice were perfused in a Langendorff apparatus, flash-frozen in liquid nitrogen, and homogenized at 4°C with a Polytron PT20 homogenizer in 2 ml buffer of the following composition (in mM): 250 sucrose, 1 EDTA, 1 PMSF, and 1 iodoacetamide. The homogenate was centrifuged at 1,000 g for 10 min, and the pellets were discarded. Each supernatant was centrifuged at 100,000 g for 1 h at 4°C, and each pellet was resuspended in 200 µl buffer containing (in mM) 20 Tris, 1 EDTA, 1 PMSF, 1 iodoacetamide, and 1/5 SDS. Proteins (60 µg of total protein) were separated by 10% SDS-PAGE and transferred to nitrocellulose membranes (Bio-Rad Laboratories). Membranes were then incubated overnight at 4°C with rabbit polyclonal primary antibodies against Kv4.2, Kv4.3, or Kv1.5 (Kv4.2 and 4.3, 1:2,000; Kv1.5, 1:100 dilution, from Upstate), followed by incubation for 90 min at room temperature with donkey anti-rabbit secondary antibody (1:2,000). Membranes were developed by using enhanced chemiluminescence and exposed to X-ray film. Quantitation was performed by scanning the blots and using Bio-Rad Quantity One for densitometry measurements.

**Statistics.** Data are expressed as means ± SE; n represents the number of cells included in the analysis and is followed by the number
Fig. 1. Separation of Ca\textsuperscript{2+}-independent depolarization-activated outward K\textsuperscript{+} currents in myocytes from adult mouse left ventricle. Whole cell outward K\textsuperscript{+} currents were evoked by 4.5-s depolarizing voltage steps to potentials between −40 and +50 mV in 10-mV increments at 15-s intervals from a holding potential of −90 mV; each voltage step was preceded by a brief depolarization of 20 ms to −20 mV to eliminate voltage-gated sodium currents not blocked completely by tetrodotoxin (TTX). After the total outward K\textsuperscript{+} current was recorded (A), myocytes were exposed to 50 μM 4-aminopyridine (4-AP; B) and the 4-AP-sensitive K\textsuperscript{+} current I\textsubscript{K,slow1} (D) was obtained by offline subtraction of the currents recorded in the presence of 50 μM 4-AP (B) from control currents (A). C: family of outward K\textsuperscript{+} currents obtained from cells perfused with 50 μM 4-AP, but voltage steps were each preceded by a prepulse (100 ms at +40 mV) to inactivate the transient outward K\textsuperscript{+} current (I\textsubscript{to}) (the voltage protocol is shown in inset). The prepulse-inactivated K\textsuperscript{+} current I\textsubscript{to} (E) was obtained by offline subtraction of the currents elicited with the prepulse (C) from the currents recorded with a normal 4.5-s-long voltage protocol (voltage protocol is shown in inset) (B). I\textsubscript{to} is shown in an extended time scale (E, inset).
of mice from which the cells were taken. Differences between membrane currents in TNF and control mice were evaluated using Student’s t-test and considered statistically significant at \( P < 0.05 \).

**RESULTS**

_Capacitance is increased in myocytes from TNF mice._ Cell membrane capacitances of ventricular myocytes isolated from left ventricular free wall of TNF mice were increased compared with control mice (190.9 ± 5.9 pF, \( n = 35 \) cells from 8 mice vs. 175.3 ± 5.6 pF, \( n = 48 \) cells from 9 mice; \( P < 0.05 \)). This is consistent with the observed hypertrophy seen in the hearts and in single ventricular myocytes from TNF mice (25, 31). Other investigators have also reported enlarged cell size as measured by capacitance in mouse models of heart failure (30, 40, 44, 65, 66).

_K⁺ currents are decreased in TNF mice._ Total K⁺ currents were measured in ventricular myocytes isolated from TNF-α and control littermate mice (Fig. 2A). On depolarization, both TNF and control myocytes exhibited a complex rapidly activating current that decayed to a nonzero steady-state level after 4.5 s. The outward currents of both TNF and control myocytes activated at −30 mV and the density of the peak current in control myocytes were similar to that reported by others in mouse myocytes (56, 64). The magnitude of the total K⁺ current in the TNF mice was markedly lower than in control myocytes over a broad range of potentials (at +40 mV, 46.2 ± 2.5 pA/pF, \( n = 14 \), 4 mice vs. 72.5 ± 3.1 pA/pF, \( n = 15 \), 4 mice; \( P < 0.001 \); Fig. 2B).

Total currents were fit to a double-exponential function (Table 1). Both the fast and slow components of the K⁺ currents were significantly decreased in the TNF compared with control mice, suggesting that both \( I_\text{fast} \) and \( I_\text{slow} \) were decreased. The time constant of \( I_\text{slow} \) was longer in myocytes from the TNF mice, suggesting differential regulation of its underlying molecular components (Kv1.5 and Kv2.1). There was no significant difference in \( I_\text{ss} \), the steady-state component of the current.

We next proceeded to dissect the role of each molecular component of the outward K⁺ current in the reduction of the total K⁺ current in TNF and control mice. There was no difference in the threshold of activation (−20 mV) of \( I_\text{K,slow1} \), the rapidly activating slowly inactivating 4-AP-sensitive current encoded by Kv1.5 (Fig. 3A). Above −10 mV, the density of \( I_\text{K,slow1} \) in TNF myocytes was significantly smaller than in controls (Fig. 3B). The mean \( I_\text{K,slow1} \) density measured at +40 mV was 22.6 ± 1.7 pA/pF in control (\( n = 17 \), 4 mice) and 12.5 ± 1.2 pA/pF in TNF (\( n = 14 \), 4 mice) myocytes (\( P < 0.001 \)).

\( I_\text{K,slow} \), the rapidly activating and inactivating K⁺ current encoded by Kv4.3 and Kv4.2, was identified as the current inactivated by a 100-ms prepulse to +40 mV in the presence of 4-AP.

**Table 1. K⁺ currents and time constants in wild-type and TNF mice**

<table>
<thead>
<tr>
<th>Mice</th>
<th>Currents at +40 mV</th>
<th>Wild Type</th>
<th>TNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>( I_\text{fast} ) pA/pF</td>
<td>72.5 ± 3.1</td>
<td>46.2 ± 2.5</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>( I_\text{ss} ) pA/pF</td>
<td>27.4 ± 1.3</td>
<td>17.8 ± 1.3</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>( \tau_\text{fast} ), ms</td>
<td>81 ± 5</td>
<td>90 ± 6</td>
<td>( P = \text{NS} )</td>
</tr>
<tr>
<td>( I_\text{K,slow} ) pA/pF</td>
<td>37.3 ± 2.5</td>
<td>20.9 ± 1.6</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>( \tau_\text{slow} ), ms</td>
<td>988 ± 30</td>
<td>1249 ± 45</td>
<td>( P &lt; 0.001 )</td>
</tr>
<tr>
<td>( I_\text{Isc} ) pA/pF</td>
<td>7.9 ± 0.4</td>
<td>7.3 ± 0.5</td>
<td>( P = \text{NS} )</td>
</tr>
</tbody>
</table>

Data are means ± SE. All currents are normalized to the cell capacitance and presented as pA/pF. Individual currents were obtained by fitting the current elicited by a 4.5-s depolarizing step to +40 mV to a double-exponential function \( I(t) = A_\infty + A_\text{fast} \exp(-t/\tau_\text{fast}) + A_\text{slow} \exp(-t/\tau_\text{slow}) \), where \( A_\infty \), \( A_\text{fast} \), and \( A_\text{slow} \) represent the amplitudes of the steady-state current (\( I_\text{ss} \)), the transient outward current (\( I_\text{fast} \)), and the slowly inactivating component of the outward current (\( I_\text{K,slow} \)), respectively. \( \tau_\text{fast} \), maximum peak outward current; \( \tau_\text{slow} \), the time constant of inactivation of \( I_\text{fast} \); \( \tau_\text{slow} \), the time constant of inactivation of \( I_\text{K,slow} \); NS, not significant.

Fig. 2. Heart failure reduces total outward K⁺ current. A: representative families of currents recorded from a holding potential of −90 mV in response to voltage steps of 4.5 s from −40 to +50 mV in 10-mV increments at 15-s intervals in control wild-type myocytes (WT; top) and TNF myocytes (bottom). B: total peak K⁺ current-voltage (I/V) relations for cells from TNF mice (squares, \( n = 14 \)) and control wild-type mice (triangles, \( n = 15 \)).
50 μM 4-AP in myocytes isolated from the left ventricular free wall of both control and TNF mice (Fig. 4A; at +50 mV: \( \tau_{\text{act}} = 1.4 \pm 0.1 \text{ ms}, \tau_{\text{inact}} = 44.5 \pm 1.6 \text{ ms}, n = 16 \) in control myocytes; \( \tau_{\text{act}} = 1.1 \pm 0.1 \text{ ms}, \tau_{\text{inact}} = 50.4 \pm 1.1 \text{ ms}, n = 17 \) in TNF myocytes). The amplitude of \( I_{\text{to,s}} \) was significantly attenuated in TNF compared with control myocytes (Fig. 4B and C). The mean \( I_{\text{to,s}} \) density measured at +40 mV was 38.3 ± 1.9 pA/pF in control (\( n = 17, 4 \) mice) and 18.6 ± 1.4 pA/pF in TNF (\( n = 18, 4 \) mice) myocytes (\( P < 0.001 \)). \( I_{\text{to,s}} \) was a more slowly inactivating component encoded by Kv1.4, is found only in myocytes isolated from the septum in control myocytes (64). The absence of a change in \( \tau_{\text{inact}} \) in myocytes from the TNF mice (and also in \( \tau_{\text{fast}} \) in Table 1) argues against an upregulation of \( I_{\text{to,s}} \).

After TNF and control cells were treated with 4-AP and the prepulse protocols, a 4-AP-resistant component remained characterized by slow activation and inactivation (Fig. 5A). The decay phase of this current was best fitted by a single exponential function that revealed a noninactivating component \( I_{\text{st}} \) and a very slowly inactivating component that consists mainly of \( I_{\text{K,slow2}} \). There was only a moderate reduction of this component in TNF mice (Fig. 5B). The mean density at +40 mV was 13.8 ± 0.7 pA/pF in control (\( n = 13, 4 \) mice) and 10.3 ± 0.7 pA/pF in TNF (\( n = 18, 4 \) mice) myocytes (\( P < 0.001 \)). This may represent a small decrease in \( I_{\text{K,slow2}} \) in myocytes from TNF mice, although we cannot exclude contamination of this current by residual \( I_{\text{K,slow1}} \). No differences were detected in the magnitude of the noninactivating component \( I_{\text{st}} \) (Fig. 5C). At +40 mV, the mean \( I_{\text{st}} \) density was 6.9 ± 0.3 pA/pF in control (\( n = 13 \)) and 6.8 ± 0.4 pA/pF in TNF (\( n = 18 \)) myocytes (\( P = \text{not significant (NS)} \)).

**L-type Ca\(^{2+}\) currents.** Robust L-type Ca\(^{2+}\) currents were present in ventricular myocytes of TNF and control mice (Fig. 6A). Measurements of Ca\(^{2+}\) currents showed no difference in the peak \( I_{\text{Ca,L}} \) density between control (−9.4 ± 0.4 pA/pF, \( n = 31, 5 \) mice) and TNF (−9.3 ± 0.5 pA/pF, \( n = 20, 4 \) mice) myocytes (\( P = \text{NS} \)). Similarly, there were no differences in the \( I_{\text{Ca,L}} \) density over the range of potentials from −10 mV to +50 mV (Fig. 6B). At −30 mV and −20 mV, the mean \( I_{\text{Ca,L}} \) density was somewhat larger in TNF compared with control myocytes. At −30 mV, \( I_{\text{Ca,L}} \) was −0.5 ± 0.07 pA/pF in TNF vs. −0.3 ± 0.03 pA/pF in control myocytes (\( P < 0.01 \)). At −20 mV, \( I_{\text{Ca,L}} \) was −2.0 ± 0.2 pA/pF in TNF vs. −1.4 ± 0.2 pA/pF in control myocytes (\( P < 0.05 \)). Fast and slow inactivation time constants (\( \tau_{\text{f}} \) and \( \tau_{\text{i}} \)), on the other hand, were similar in TNF myocytes and controls. At 0 mV, \( \tau_{\text{f}} \) was 20.5 ± 1.2 ms in TNF vs. 20.2 ± 0.9 ms in control myocytes, and \( \tau_{\text{i}} \) was 85.1 ± 5.2 ms in TNF vs. 83.6 ± 5.3 ms in control myocytes. In addition, no significant differences were present in the deactivation kinetics of the Ca\(^{2+}\) current, measured via the time constant of inactivation (\( \tau \)) from a single-exponential fit to the decaying tail current after strong depolarizing steps. At +50 mV, the time constant of inactivation of the tail current \( \tau \) was 7.6 ± 4.3 ms in TNF vs. 6.4 ± 1.8 ms in control myocytes, and at +30 mV, \( \tau \) was 5.5 ± 1.4 ms in TNF vs. 7.1 ± 1.7 ms in control myocytes (\( P = \text{NS} \)).

**Western blot analysis of K\(^{+}\) channel proteins.** Western blot analysis revealed that the protein levels of Kv4.2 and Kv4.3 were reduced by 80% and 48%, respectively, in membrane fractions isolated from the hearts of TNF mice compared with controls (Fig. 7A). This is consistent with the observed decrease in \( I_{\text{to,f}} \). Similarly, protein levels of Kv1.5 were also reduced by 43%, consistent with the observed decrease in \( I_{\text{K,slow1}} \) (Fig. 7B).

**DISCUSSION**

**K\(^{+}\) currents in TNF mice.** Outward K\(^{+}\) currents in the heart are responsible for repolarization of the membrane potential, and K\(^{+}\) channel expression varies significantly by species (41). Mouse ventricular myocytes express both transient outward and delayed rectifier K\(^{+}\) currents. The transient outward K\(^{+}\) currents have rapid activation and inactivation. They include \( I_{\text{to,f}} \) encoded by the Kv4.2 and Kv4.3 genes and \( I_{\text{to,s}} \) encoded by the Kv1.4 gene (20, 64). \( I_{\text{to,s}} \) currents are identified predominantly in septum cells and have slower inactivation kinetics...
and recovery from inactivation than $I_{\text{to,f}}$. The delayed rectifier currents in mouse ventricle activate rapidly and inactivate slowly. They include the 4-AP-sensitive $I_{\text{K,slow1}}$ encoded by the Kv1.5 gene and the TEA-sensitive $I_{\text{K,slow2}}$ encoded by the Kv2.1 gene (64). In the mouse ventricle there is also a noninactivating outward K$^+$ current referred to as $I_{\text{ss}}$. Our whole cell voltage-clamp recordings from left ventricular myocytes of TNF mice revealed a decrease (at +40 mV) in the current densities of 51% for $I_{\text{to,f}}$, 45% for $I_{\text{K,slow1}}$, and 25% for $I_{\text{K,slow2}}$. These changes were consistent with the decrease in the protein expression level of Kv4.2 and Kv1.5 as revealed by Western blot analysis, although the findings are limited by the fact that the electrophysiological studies were performed on left ventricular free wall myocytes, whereas the protein studies were performed on whole heart homogenates. Thus it seems likely that the decrease in K$^+$ currents that we observed in ventricular myocytes from the TNF mice at least partially contributes to the increase in APD that we measured previously using optical mapping (36).

Electrophysiological studies have been performed on a number of transgenic mouse models of hypertrophy and heart failure, although measurements of K$^+$ currents have focused on the transient outward current $I_{\text{to}}$, and a complete description of the individual repolarizing currents is absent (6, 11, 30, 40, 44, 61). Mice with systolic dysfunction due to overexpression of calsequestrin, the G protein Gq, or a dominant negative fragment of the K$^+$ channel Kv4.2 have decreased densities of multiple repolarizing K$^+$ currents, including $I_{\text{to}}$, a slowly or noninactivating component $I_{\text{sustained}}$, and the inward rectifier current $I_{\text{K1}}$ (30, 40, 61). Mice overexpressing calcineurin or the L-type Ca$^{2+}$ channel develop a decrease in $I_{\text{to}}$ only at ages over 3 or 9 mo, respectively, when frank heart failure begins to develop (6, 44). Transgenic mice overexpressing fatty acid transport protein 1 (FATP1) develop diastolic dysfunction.

Fig. 4. Heart failure reduces $I_{\text{to}}$. A: family of K$^+$ currents from a control cell obtained by subtracting corresponding current records with and without the inactivating prepulse shown in an extended time scale. The chosen scale shows clearly the rapid activation and inactivation kinetics of the current. B: representative families of $I_{\text{to}}$ obtained by subtracting corresponding currents with and without the inactivating prepulse in control wild-type cells (top) and TNF cells (bottom). C: mean I/V relations for $I_{\text{to}}$ in cells from TNF mice (squares) and control wild-type mice (triangles).
associated with a decrease in $I_{K,slow}$ and no change in $I_{to,t}$ (11). Here, in the TNF mouse model of heart failure, we show significant decreases in $I_{to,f}$, $I_{K,slow1}$, and $I_{K,slow2}$, with no change in $I_{ss}$. Thus downregulation of $I_{to,t}$ is a consistent finding in mouse models of systolic dysfunction.

The action potential prolongation that results from $K^+$ channel inhibition may contribute to early afterdepolarizations and triggered activity (19). Decreased repolarizing currents may also affect excitation-contraction coupling and $Ca^{2+}$ release from the sarcoplasmic reticulum by modulating either $I_{Ca,L}$ or by inhibition of $Ca^{2+}$ extrusion via the Na/Ca exchanger (47). This would be especially true for changes in the early phases of repolarization, as would be mediated by alterations in $I_{to}$.

Our results showing attenuation of transient outward $K^+$ currents in the TNF mice are similar to the downregulation of $I_{to}$ in failing ventricular myocardium in patients and large animal heart failure models (4, 27, 46, 57). There are marked differences in the expression patterns of $K^+$ channels in different animal species, however. In large animals and humans, the major repolarizing currents are the delayed rectifiers encoded by KvLQT1/mink and Erg (41). Myocytes from humans and large animals with heart failure do not consistently show downregulation of these delayed rectifier currents (4, 27).

Although the TNF mice have decreased $I_{to,t}$ and $I_{K,slow1}$ associated with APD prolongation and arrhythmias, optical mapping studies failed to show a change in the spatial dispersion of repolarization or refractoriness between the apex and the base of the heart (36). In contrast, transgenic mice overexpressing a truncated Kv1.x $\alpha$-subunit (Kv1DN mice) lack

Fig. 5. Heart failure reduces the rapidly activating, slowly inactivating delayed rectifier $K^+$ current $I_{K,slow2}$ moderately and does not change the steady-state current $I_{ss}$. $A$: representative traces of $K^+$ currents evoked by 4.5-s depolarizing voltage steps to potentials between $-40$ and $+50$ mV preceding by an inactivating prepulse in cells perfused with 50 $\mu$M 4-AP from control wild-type mice (top) and TNF mice (bottom). $B$: mean $I/V$ relations for $I_{K,slow2}$ in cells from TNF mice (squares) and control wild-type mice (triangles). $C$: mean $I/V$ relations for $I_{ss}$ in cells from TNF mice (squares) and control wild-type mice (triangles).

Fig. 6. Heart failure does not affect L-type $Ca^{2+}$ currents. $A$: family of L-type $Ca^{2+}$ currents elicited by 250-ms depolarizing voltage steps to potentials between $-40$ and $+60$ mV in 10-mV increments at 5-s intervals in control cells (top) and cells isolated from TNF mice (bottom). Each voltage step was preceded by a short depolarization (50 ms to $-45$ mV) to eliminate contamination from sodium current ($I_{Na}$), not blocked completely by TTX. $B$: averaged peak $Ca^{2+}$ $I/V$ relations for cells from TNF mice (squares) and control wild-type cells (triangles).
IK,slow1 and have spontaneous and inducible arrhythmias due to enhanced spatial dispersion of repolarization and refractoriness between the apex and base (2, 37). Dominant negative transgenic mice overexpressing a point mutation of the Kv4.2 α-subunit (Kv4DN mice) lack I_{K,slow} and have APD prolongation without spontaneous ventricular arrhythmias (3). Transgenic mice overexpressing a mutant Kv2.x α-subunit lack I_{K,slow1} and have APD prolongation along with both spontaneous and inducible arrhythmias (63). By crossbreeding Kv1DN and Kv4DN mice, Brunner et al. (8) produced mice with a functional knockout of both I_{K,slow} and I_{K,slow1}. Although these mice had marked prolongation of APD, they were less prone to arrhythmias than the Kv1DN mice. These data suggest that while reduction in K+ currents like the ones we observed in TNF mice may contribute to the development of ventricular arrhythmias, other factors are likely to also be involved.

Ca2+ currents in TNF mice. L-type Ca2+ channels are the primary source of Ca2+ entry in the cell to trigger release from the sarcoplasmic reticulum and activate contraction, and they contribute to maintenance of the action potential plateau. Our data show no significant differences in either the peak amplitude or the inactivation kinetics of I_{Ca,L} in cardiac myocytes from TNF mice compared with controls. The small increase in peak I_{Ca,L} amplitude at potentials near the activation threshold might reflect I_{Ca,L} contamination despite the presence of 0.010 mM TTX and the depolarization steps. Thus changes in the amplitude of the Ca2+ currents do not appear to be responsible for APD prolongation or decreased contractility in the TNF mice. We cannot completely exclude the possibility that the smaller Ca2+ transient in the TNF myocytes (36) leads to less Ca2+-induced inactivation of the Ca2+ current and longer APDs in vivo, however, because the patch-clamp studies were performed by using intracellular dialysis with high concentrations of EGTA.

A number of other mouse models of dilated cardiomyopathy and heart failure also show no change in the amplitude and kinetics of I_{Ca,L}, including LIM protein knockout mice, Coxsackievirus B3 transgenic mice, and Goα-overexpressing mice (14, 40, 60). In contrast, mice overexpressing calsequestrin show significantly smaller peak I_{Ca,L} with slowed inactivation compared with controls (30, 49), whereas mice overexpressing calcineurin have increased peak I_{Ca,L} amplitude and more rapid activation kinetics (44, 66). These differences probably result from the direct effects of calsequestrin and calcineurin overexpression on intracellular calcium signaling and regulation. In large animal models and patients with heart failure, either no change or decreases in the density of I_{Ca,L} have been reported (45).

The TNF mouse model of heart failure. TNF-α has been thought to play a role in heart failure because 1) serum levels are elevated and correlate to severity of heart failure in humans (54), 2) the failing human heart synthesizes TNF-α whereas the normal heart does not (55), and 3) the myocardium expresses both forms of TNF receptors (TNFR1 and TNFRII; Ref. 55). In addition, exposure to TNF-α depresses contractile properties of isolated cardiac cells, muscles, and hearts in vitro and in vivo; induces cardiomyocyte hypertrophic growth and cardiac myocyte apoptosis; and alters calcium transient kinetics (15). To ascertain the potential for chronic TNF-α exposure to produce a disease state resembling congestive heart failure, our laboratory and others have created transgenic mice with cardiac-restricted overexpression of TNF-α (9, 31, 34). These mice develop many pathophysiological characteristics consistent with human heart failure, including increased mortality, cardiac hypertrophy and dilation, pulmonary congestion, cardiac fibrosis, arrhythmias, diminished developed pressures, and depressed systolic function (28, 31, 36). The mice have been used to study the mechanisms underlying heart failure and its progression, including increased expression of matrix metalloproteinases, slower calcium transient kinetics, increased expression of inducible nitric oxide synthase, increased cardiac cell apoptosis, reduction of adrenergic responsiveness, prolonged APD, and expression of a cardiac fetal gene profile (17, 25, 32, 35). Although the molecular mechanisms by which these alterations occur are probably multifaceted and only incompletely described, they likely are mediated through the TNFRI receptor (23) and may involve increased production of reactive oxygen species (38) and increased activation of the transcriptional regulator nuclear factor-kB (32). Thus the TNF mouse provides a relevant model to examine the molecular mechanisms by which cytokines, such as TNF-α, may mediate the cardiac structural and functional remodeling that underlies heart failure.

The mechanisms by which TNF-α may influence the electrophysiological remodeling of the failing heart are less well understood. TNF blockade limits malignant ventricular tachyarrhythmias in canine infarct model, suggesting a role for TNF in generating arrhythmias (67). Some evidence points to alterations in potassium channel transcript levels, protein expression, and channel activity due to TNF-α expression. TNF-α can induce ceramide production in cardiac tissues (28), which can inhibit potassium currents in various cell types (10, 62). On the other hand, TNF-α can increase the transient outward K+...
current in cultured rat cortical neurons by a phosphatidylincholine-specific phospholipase C and protein kinase C-specific mechanisms (24). TNF-α inhibits the IKr current via the TNFR1 receptor in isolated canine cardiac myocytes (59). This mechanism involves reactive oxygen species and occurs without changes in HERG protein or transcript expression. Rats with autoimmune myocarditis, a TNF-dependent process (1), have increased ventricular vulnerability to arrhythmias. These rats display specific alterations in potassium channel expression, including decreased expression of Kv4.2 and Kv1.5 transcripts and proteins (48, 58). Previous microarray studies from our laboratory revealed alterations in several potassium channel transcript levels in the TNF mice, including significant downregulation of Kv4.2 and Kv2.1 RNA (53). In the current study, we confirm downregulation of the Kv4.2 protein, along with decreases in INa and IKslow2. We also show decreases in Kv1.5 protein and IKslow1. Thus TNF-α expression alters both ion channel expression and repolarizing currents and may potentially contribute to cardiac arrhythmias in a variety of heart failure models and humans.

We did not exclude alterations in spatial variations of ion channel expression or ionic currents between the endocardium and epicardium of the TNF mice. In addition, many structural and electrophysiological differences exist between the mouse and human heart and make generalization of findings to large animals and humans difficult. Despite these limitations, heart failure models, such as the TNF mouse, provide an opportunity to investigate cellular mechanisms underlying the electrophysiological changes in a system that does not primarily alter cellular electrophysiology or Ca2+ handling. The effects of additional structural, metabolic, genetic, and pharmacological manipulations can be investigated by using this model.

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