Targeted Expression of a Dominant-Negative \(K_{\text{v}}4.2\) \(K^+\) Channel Subunit in the Mouse Heart

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Abstract—Action potential duration is prolonged in many forms of heart disease, often as a result of reductions in \(Ca^{2+}\)-independent transient outward \(K^+\) currents (ie, \(I_{\text{to}}\)). To examine the effects of a primary reduction in \(I_{\text{to}}\) current in the heart, transgenic mice were generated that express a dominant-negative N-terminal fragment of the \(K_{\text{v}}4.2\) pore-forming potassium channel subunit under the control of the mouse \(\alpha\)-myosin heavy chain promoter. Two of 6 founders died suddenly, and only 1 mouse successfully transmitted the transgene in mendelian fashion. Electrophysiological analysis at 2 to 4 weeks of age demonstrated that \(I_{\text{to}}\) density was specifically reduced and action potential durations were prolonged in a subset of transgenic myocytes. The heterogeneous reduction in \(I_{\text{to}}\) was accompanied by significant prolongation of monophasic action potentials. In vivo hemodynamic studies at this age revealed significant elevations in the mean arterial pressure, peak systolic ventricular pressures, and ±dP/dt, indicative of enhanced contractility. Surprisingly, by 10 to 12 weeks of age, transgenic mice developed clinical and hemodynamic evidence of congestive heart failure. Failing transgenic hearts displayed molecular and cellular remodeling, with evidence of hypertrophy, chamber dilatation, and interstitial fibrosis, and individual myocytes showed sharp reductions in \(I_{\text{to}}\) and \(I_{\text{K1}}\) densities, action potential duration prolongation, and increased cell capacitance. Our results confirm that \(K_{\text{v}}4.2\) subunits contribute to \(I_{\text{to}}\) in the mouse and demonstrate that manipulation of cardiac excitability may secondarily influence contractile performance. (Circ Res. 1999;85:1067-1076.)

Key Words: \(K^+\) channel • transgenic • cardiac electrophysiology • mouse • heart failure

Heart disease results from a variety of primary genetic and acquired stimuli.\(^1\)--\(^6\) Regardless of the initiating cause, neurohumoral and cellular responses typically cause hypertrophic remodeling of the heart characterized by genetic, biochemical, structural, and functional alterations within myocytes.\(^7\)--\(^10\) One commonly observed feature of diseased myocardium is action potential prolongation.\(^11\)--\(^13\) Although the cardiac action potential arises from the composite activity of numerous ion channels and transporters, its prolongation correlates strongly with reductions in repolarizing \(K^+\) currents and \(K^+\) channel gene expression, particularly the \(Ca^{2+}\)-independent transient outward current (\(I_{\text{to}}\)).\(^11\)--\(^15\)

Heterogeneous action potential prolongation is associated with an enhanced propensity for cardiac arrhythmias.\(^12\)--\(^16\),\(^16\)--\(^17\) The abnormally prolonged repolarization predisposes to early and delayed afterdepolarizations and triggered activity, whereas the dispersion of refractoriness may facilitate stable reentry.\(^16\)--\(^18\) This association between action potential prolongation as a result of reduced \(K^+\) currents and arrhythmias is highlighted by the high incidence of arrhythmogenesis in acquired and inherited forms of the long-QT syndrome.\(^19\),\(^20\)

Action potential prolongation also strongly influences \([Ca^{2+}]_i\), transient magnitude.\(^21\) This effect appears to underlie the positive inotropic actions of \(\alpha\)-adrenergic receptor activation\(^22\),\(^23\) that occurs both acutely and chronically in heart disease.\(^10\) In turn, elevated \(Ca^{2+}\) may contribute to cellular hypertrophy commonly seen in heart disease by acting as a stimulus for cellular growth through the activation of a number of cell signaling pathways,\(^9\) such as the recently described calcineurin-dependent pathway.\(^24\)

In this study, we examined the phenotypic consequences of a primary reduction in repolarizing transient outward currents in the heart. We created transgenic mice overexpressing an N-terminal fragment of \(K_{\text{v}}4.2\), a strategy predicted to specifically reduce \(I_{\text{to}}\)\(^25\)--\(^29\) by a dominant-negative mechanism.\(^30\) We found that expression of the transgene was poorly tolerated; of 6 transgenic founders, 2 died suddenly, 3 others failed to achieve germline transmission, and only 1 line transmitted the transgene in mendelian fashion. In this surviving line, neonatal hearts appeared structurally normal but displayed increased contractility in vivo, and myocytes showed specific although heterogeneous reduction in \(I_{\text{to}}\)
Figure 1. K⁺ channel constructs and in vitro characterization. A, Schematic diagram of the Kᵥ4.2 protein, which contains 6 transmembrane segments (S1–S6) and a pore-forming loop between S5 and S6. A truncated N-terminal fragment of Kᵥ4.2 (Kᵥ4.2N, 2kDa) encoding the first 311 amino acids (S1–S4) of the parent molecule was generated. The truncated N-terminal construct contains those sequences that are known to be important for subtype-specific tetramerization but lacks the pore-forming P-loop. An HA epitope tag was placed in frame at the C terminus of the Kᵥ4.2N gene. B, Whole-cell voltage-clamp records show typical families of outward currents recorded in tsa201 cells transfected with full-length Kᵥ4.2 plus a 3-fold excess of vector alone (left, top) or with full-length Kᵥ4.2 plus a 3-fold excess of Kᵥ4.2N-HA (left, bottom). Kᵥ4.2 induced the expression of large, inactivating outward currents that were absent in cells cotransfected with a 3-fold excess of Kᵥ4.2N-HA. Right, Relative current densities in control cells (Kᵥ4.2 alone, open bar), in cells cotransfected with a 3-fold excess of Kᵥ4.2N-HA (solid bar), and in cells cotransfected with a 3-fold excess of a truncated Kᵥ1.4 fragment (Kᵥ1.4N, hatched bar). Data are mean±SEM of 5 to 27 experiments.

Isolation of Mouse Ventricular Myocytes
Mouse hearts were isolated and perfused in retrograde fashion with a solution of collagenase and protease using a slight modification of a procedure previously described.28

Electrical Recordings in Ventricular Myocytes, Oocytes, and tsa201 Cells
Two-electrode voltage-clamp recordings of Xenopus laevis oocytes were performed as previously described.33 Membrane currents were recorded from mammalian tsa201 and from Ca²⁺-tolerant, rod-shaped ventricular myocytes using the whole-cell configuration of the patch-clamp technique, essentially as previously described.35

Iₒ was measured as peak current elicited by the depolarizing voltage step minus the current remaining at the end of the 500-ms voltage step (ie, Iₒ). Iₒ was measured as a Ba²⁺-sensitive current using 500-ms steps from −130 to 0 mV (10-mV increments) from the holding potential in the presence and absence of 0.3 mM BaCl₂. Action potentials were elicited at a frequency of 1 Hz and were recorded in the absence of BaCl₂.

Microsurgical Methods and In Vivo Hemodynamic Measurements
Mice were anesthetized, and the carotid artery was cannulated with polyethylene tubing (PE-200), which was connected to a TD-310 low-compliance pressure transducer (MicroMed) and amplified by a blood pressure analyzer (BPA model 300, MicroMed). Heart rate, aortic pressure, left ventricular (LV) systolic pressure, LV diastolic pressure, and the maximum and minimum first derivatives of the LV pressure (+dP/dtmax and −dP/dtmax, respectively) were recorded.

Echocardiographic Assessment
Mice were anesthetized and examined by transthoracic echocardiography using a 12-MHz probe (Hewlett Packard). Ejection velocity, end-systolic (ESD), and end-diastolic (EDD) dimensions were recorded and fractional shortening (FS) was calculated as: FS=(EDD−ESD)/EDD.

Monophasic Action Potentials
Hearts were retrogradely perfused with Tyrode’s solution at 37°C, and action potentials were recorded from the surface of the left ventricles using a close-bipolar configuration.35 The times for 50% (APD₅₀) and 90% (APD₉₀) repolarization were recorded.

An expanded Materials and Methods section is available online at http://www.circresaha.org.

Results
Characterization of the Kᵥ4.2N Dominant-Negative Construct in Xenopus Oocytes
Initial experiments were designed to test the efficacy and specificity of the epitope-tagged dominant-negative Kᵥ4.2N construct, schematized in Figure 1A. Coinjection of the HA-tagged truncated Kᵥ4.2N-HA with the full-length Kᵥ4.2 into Xenopus oocytes caused a significant (P<0.01), dose-dependent reduction in current (ie, peak current amplitude was 1.13±0.13 μA [n=16], 0.25±0.04 μA [n=12], and −0.18±0.11 μA [n=7] when the ratio of Kᵥ4.2N to Kᵥ4.2 was 0:1, 1:1, and 3:1). In contrast, peak current was not affected by coinjection of a 3-fold excess of the parental pGWI1 plasmid with Kᵥ4.2 (1.11±0.09 μA)23 or coinjection of a 3- to 10-fold excess of Kᵥ4.2N-HA with Kᵥ1.4 or Kᵥ2.1 (data not shown). Similar dominant-negative inhibition was observed in tsa201 mammalian cells. Figure 1B shows typical families of outward currents recorded in cells transfected either with Kᵥ4.2 plus a 3-fold excess of vector alone (top) or with Kᵥ4.2 plus a 3-fold excess of Kᵥ4.2N-HA (bottom).
Kv4.2N-HA coexpression significantly (P<0.001) reduced current densities from 375.6 ± 84 pA/pF (n = 17) to only 7.9 ± 6.6% of control (n = 6). In contrast, a 3-fold excess of Kv4.2N-HA did not affect Kv1.4 current densities. Collectively, these results establish that the Kv4.2N-HA fragment potently and specifically inhibits Kv4.2-based currents, as expected from previous results.

### Generation and Initial Characterization of Transgenic Mice

Six founders harboring the Kv4.2N transgene were initially identified from a total of 34 live births screened. Two of these founders died suddenly before yielding any progeny and were discarded without further analysis. Three additional founders produced multiple litters with no transgenic offspring. The sixth founder yielded several litters with a normal distribution of transgenic progeny from which a line was established and then died suddenly as well. These initial observations of the F0 transgenic mice strongly suggested that expression of the dominant-negative Kv+ channel subunit in the heart was deleterious.

Transgene expression in the hearts of mice from the established line was examined at both the transcript and protein levels, as illustrated in Figure 2A. Northern blot analysis of cardiac RNA revealed abundant accumulation of the MHCα-Kv4.2N transcript (left). Western blot analysis using an antibody directed against an amino-terminal epitope of Kv4.2 (Kv4.2 Ab) detected expression of the endogenous Kv4.2 protein in both control and transgenic hearts, whereas the truncated HA-tagged protein was expressed exclusively in transgenic hearts (right). The identity of the truncated Kv4.2N protein was confirmed by immunoblotting with a monoclonal antibody directed against the HA epitope tag. The level of Kv4.2N expression remained unchanged from 3 weeks to 12 weeks of age (Figure 2B).

### Clinical Course

Although transgenic mice initially appeared healthy and indistinguishable from nontransgenic littermates, by 12 to 13 weeks of age most transgenic mice developed obvious signs of congestion consistent with biventricular heart failure. Mice at this stage were dyspneic and sedentary and frequently appeared edematous, as illustrated in Figure 3A. Hearts isolated from transgenic mice at 13 weeks were enlarged (Figure 3B and 3C), and heart weight/body weight ratios were significantly increased compared with littermate controls, as summarized in Table 1. On cross section, ventricular hypertrophy and chamber dilatation, especially in the left atrium, was observed (Figure 3D through 3F). There was also evidence of myocyte hypertrophy, myocyte cell loss, and interstitial fibrosis and cellularity (Figure 3G through 3I). A markedly dilated left atrium with organized thrombus formation was observed in virtually all transgenic hearts.
tion was observed in essentially all transgenic mice at this stage (Figure 3J).

Phenotypic Characterization of 2- to 3.5-Week-Old Transgenic Mice

To begin to understand the time course of disease progression in the MHCα-K,4.2N transgenic mice, we first characterized cardiac morphology, gene expression, ionic currents, and hemodynamic properties in neonatal preparations. At 2 to 3.5 weeks of age, despite robust transgene expression (Figure 2), there was no overt evidence of cardiac hypertrophy (see Table 1). Heart weights and heart weight/body weight ratios were indistinguishable from those of nontransgenic littermates. There was a modest increase in ventricular ANF expression but no significant alterations in other traditional markers of cardiac hypertrophy, including SERCA and PLB, as summarized in Table 2. In fact, average myocyte membrane capacitance (Cm) was significantly smaller (P<0.04) in transgenic myocytes (Cm=87.0±3.8 pF, n=33) compared with nontransgenic control cells (Cm=107.5±4.8 pF, n=27), consistent with the absence of gross cardiac enlargement.

We next examined the electrophysiological properties of myocytes from young transgenic mice and nontransgenic littermates. Figure 4A shows representative current density traces in right ventricular myocytes from 23-day-old control (left) and transgenic (center and right) littermates; Figure 4B shows the corresponding current-voltage relationships of the peak current (I0) and the current remaining at the end of the 500-ms pulse (I1/2) for the same cells. The difference between the peak outward current and the current remaining at the end of the 500-ms pulse was defined as the transient outward current (I0). The frequency histogram of I0 shown in Figure 4C demonstrates uniformly high current densities in control myocytes, with only 11.5% of these cells exhibiting I0 densities below 40 pA/pF. In contrast, transgenic myocytes displayed a much broader range of I0 densities, and almost half (45.4%) exhibited I0 densities below 40 pA/pF. Statistical comparisons based on χ2 tests (see Materials and Methods) revealed that the 2 distributions were statistically distinct (P<0.05). Moreover, bivariate normal distribution functions gave statistically better fits (P<0.05, F statistics) to the current amplitude distribution data than a monovariate function in the transgenic mice but not in the control mice. These observations suggest that the current densities in transgenic mice comprise 2, or possibly more, populations of cells (see Discussion). Overall, at this age, I0 current densities were significantly (P<0.05) reduced in transgenic myocytes (48.7±5.4 pA/pF, n=33) compared with control myocytes (61.2±4.3 pA/pF, n=26), as assessed using the nonparametric Kolmogorov-Smirnov method. Identical observations were made from myocytes isolated from the left ventricle, where I0 measured at +60 mV was significantly (P<0.02) reduced from 57.4±4.3 pA/pF (n=22) in control hearts to 39.6±3.1 pA/pF (n=25) in transgenic hearts.

Next, we investigated whether changes in I0 were associated with alterations in action potential duration (APD). Control action potentials were typically short and showed no evidence of a plateau (Figure 4D, left). On average, control APD50 and APD90 values were 3.5±0.16 ms (n=12) and 20.6±2.1 ms (n=12). As with I0, some myocytes from young transgenic mice had spiked action potentials that were indistinguishable from those of control cells (Figure 4D, center), whereas other myocytes had markedly prolonged action potentials that were never seen in controls (Figure 4D, right).

Importantly, the myocytes with reduced I0 invariably had prolonged action potentials, consistent with I0 reduction being responsible for APD prolongation. Despite mixed populations of myocytes in transgenic mice, the average APD50 and APD90 values were significantly (P<0.03) prolonged in transgenic mice compared with control mice. Consistent with these observations, we found that monophasic action potentials recorded from the apical LV epicardium of transgenic hearts were significantly prolonged at both 50% and 90% of repolarization, as shown in Figure 5. APD50 was 12.3±1.0 ms in control versus 49.2±8.3 ms in transgenic hearts (P<0.003), and APD90 was 53.5±5.9 ms in control versus 108.7±11.0 ms in transgenic hearts (P<0.005), establishing that global changes in repolarization occur in young transgenic mice. Despite changes in APD, no differences in resting membrane potential between the groups (−83.8±0.9

### Table 1. Morphological Properties of Mouse Hearts

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<th>3 Weeks</th>
<th>12 to 14 Weeks</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Transgenic</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>10.4±0.4 (14)</td>
<td>10.5±0.6 (8)</td>
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<tr>
<td>Heart weight, mg</td>
<td>68±3 (14)</td>
<td>68±4 (8)</td>
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<tr>
<td>Heart/body, mg/g</td>
<td>6.6±0.2 (14)</td>
<td>6.5±0.2 (8)</td>
</tr>
<tr>
<td>Lung/body, mg/g</td>
<td>7.3±0.3 (5)</td>
<td>7.1±0.4 (4)</td>
</tr>
<tr>
<td>Liver/body, mg/g</td>
<td>46.1±0.4 (5)</td>
<td>43.6±0.5 (4)</td>
</tr>
</tbody>
</table>

*Number of individual mice studied is shown in parentheses.

### Table 2. Gene Expression

<table>
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<tr>
<th></th>
<th>3 Weeks</th>
<th>12 to 14 Weeks</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Transgenic</td>
</tr>
<tr>
<td>ANF</td>
<td>1±0.23 (5)</td>
<td>2.74±0.48* (6)</td>
</tr>
<tr>
<td>SERCA</td>
<td>1±0.11 (6)</td>
<td>1.55±0.23 (6)</td>
</tr>
<tr>
<td>PLB</td>
<td>1±0.07 (5)</td>
<td>1.26±0.23 (6)</td>
</tr>
<tr>
<td>Kv4.2</td>
<td>1±0.14 (5)</td>
<td>1.30±0.11 (6)</td>
</tr>
</tbody>
</table>

*Number of individual mice studied is shown in parentheses.

*P<0.05 between age-matched transgenic and nontransgenic mice.
mV \( [n=12] \) and \(-83.7\pm0.7\) mV \( [n=16] \), in control and transgenic cells, respectively) as expected from the \( I_{K1} \) results (see Figure 6).

To examine the specificity of the K\(_{4.2N}\)-dependent reduction of \( I_{to} \), several additional K\(_1\) currents were examined. \( I_{500} \) (recorded at the end of a 500-ms depolarization step) and the distribution histograms were no different (\( P>0.1 \)) in right ventricular myocytes from 2- to 3.5-week-old transgenic compared with control mice (\( I_{500}=24.4\pm1.3 \) pA/pF \( [n=26] \) control versus \( 21.5\pm1.2 \) pA/pF \( [n=31] \) transgenic). Similar results were observed in myocytes isolated from the left ventricle (not shown). Furthermore, \( I_{K1} \) densities were not significantly different between transgenic cells exhibiting \( I_{to} \) densities \(<40\) pA/pF (\( I_{K1} \) density \( =-15.5\pm2.1 \) pA/pF, \( n=9 \)) and control cells (\(-15.7\pm1.4, n=13 \)) or transgenic cells exhibiting an \( I_{to} \) density above \( 40 \) pA/pF (\(-16.4\pm0.6 \) pA/pF, \( n=10 \)), as illustrated in Figure 6. As expected, an ANCOVA on the distributions summarized in Figure 4C confirmed that \( I_{K1} \) densities did not correlate with \( I_{to} \) (\( P>0.6 \)) (see online supplementary information for a tabulation of all electrophysiological parameters; http://www.circresaha.org).

We next examined the hemodynamic characteristics of these young mice. The mean aortic pressure and peak systolic ventricular pressures were significantly elevated in transgenic mice compared with age-matched control littersmates. More notable were the significant increases in the magnitude of both \(+dP/dt\) and \(-dP/dt\) in the transgenic mice versus littermate controls. Neither heart rates nor end-diastolic pressures were significantly different between the groups, suggesting that the differences in \( dP/dt\) between the groups were not due to changes in preload. These differences in hemodynamic parameters are not the result of the limited frequency response of our pressure recording system, because signal filtering will tend to reduce (not enhance) the observed differences. The elevated contractility coincided with significant increases in both peak LV ejection velocities and fractional shortening, as measured by echocardiography using Doppler and M-mode recordings, respectively. These results, summarized in Table 3, establish unequivocally that contractility was elevated in the transgenic mice with reduced \( I_{to} \) and global action potential prolongation, before the development of overt hypertrophy.
systolic ventricular pressures, increased LV end-diastolic pressures, elevated heart rates, and severe depression of both +dP/dt and –dP/dt. Consistent with these hemodynamic changes, echocardiographic studies revealed that adult transgenic mice displayed significant reductions in LV fractional shortening and peak aortic injection velocities, as summarized in Table 3.

In contrast to the findings in young transgenic mice, the average myocyte membrane capacitance (C_M), estimated in patch-clamp experiments, was significantly (P<0.05) greater in transgenic cells (232±16 pF, n=16) compared with control cells (156±6 pF, n=23). Figure 7 shows representative current density traces (Figure 7A) and average current-voltage relationships (Figure 7B) recorded in control (left) and transgenic (right) right ventricular myocytes. Whereas I_Na was activated over the same range of voltages between the 2 groups, the average current densities at +60 mV were reduced in transgenic (16.5±3.9 pA/pF, n=11) compared with control cells (48.4±3.4 pA/pF, n=21). As expected from the reductions in outward K+ currents, action potentials were typically prolonged in myocytes isolated from the transgenic mice compared with age-matched control mice (Figure 7C). On average, APD50 and APD90 values were significantly (P<0.05) different between control (3.8±1.0 ms, n=17) vs control hearts (solid bars). Data are mean±SEM.

Phenotypic Characterization of Adult Mice

By 3 to 4 months of age, most transgenic mice developed clinical signs of congestive heart failure accompanied by gross and histological evidence of a cardiomyopathy (Figure 3). Hemodynamic studies confirmed this impression. Compared with littermate controls, transgenic mice had significantly (P<0.05) reduced peak aortic pressures, reduced peak

Figure 5. Monophasic action potential recordings. A, Typical monophasic action potential recordings from isolated hearts from control (left) and transgenic (right) mice. B, On average, APD50 and APD90 were significantly (*P<0.01) longer in the transgenic mouse hearts (hatched bars) vs control hearts (solid bars). Data are mean±SEM.

Figure 6. Inward rectifier currents in 2- to 3.5-week-old mice. A, Representative families of repolarizing K+ currents recorded from myocytes isolated from 2- to 3.5-week-old control (left) and transgenic (right) mice. Currents were elicited by 500-ms steps to voltages in the range −130 to 60 mV (in 10-mV increments from −130 to −60 mV and then in 20-mV steps to 60 mV). I_K1 densities were similar in the control and the transgenic cells, despite the fact that I_K1 was absent in the transgenic cells. B, I_K1 density in control current (□) and transgenic (■) cells were determined at −130 mV and plotted against I_Na density (at 60 mV) in the same cells. Cells below the dashed horizontal line and to the right of the dashed vertical line express low I_Na density and low I_K1 density, respectively (see text for explanation). ANCOVA of the distributions confirmed that low I_K1 densities were not predictors of the density of I_Na.

Discussion

Previous studies in animal models and human patients have established that reductions in the Ca2+-independent transient
transgenic lineages harboring the K\(_{\text{v}}\)4.2N transgene was deleterious phenotype with Kv\(_{\text{v}}\)4.2 truncation mutants (G. Tomaselli, personal communication, November 1998), suggesting that expression of the truncated K\(_{\text{v}}\)4.2-GFP fusion construct 51 as well as the endogenous full-length protein, may induce a cardiomyopathy in these mice which is expressed at levels 52 have both demonstrated abnormal trapping of the ectopically expressed mutant K\(_{\text{v}}\)4.2N polypeptide. Recent studies have demonstrated that overexpression of foreign proteins can induce an endoplasmic reticular (ER) stress response, resulting in activation of a number of cell signaling and kinase pathways such as C/EBP homologous protein (CHOP) and the induction of apoptosis.49,50 Consistent with this mechanism, previous studies in cultured myocytes expressing a truncated K\(_{\text{v}}\)4.2-GFP fusion construct 51 as well as GH3 pituitary cells expressing a truncated K\(_{\text{v}}\)1.1 polypeptide 52 have both demonstrated abnormal trapping of the mutant protein in the ER. Indeed, Huang and Izumo 53 have recently described a cardiomyopathy in transgenic mice expressing high levels of a “biologically inert” protein. Thus, it is conceivable that the truncated Kv4.2N polypeptide, which is expressed at levels \(\approx \)10-fold greater than the endogenous full-length protein, may induce a cardiomyopathy by interference with normal cell trafficking and the induction of an ER stress response. Consistent with this alternative mechanism, Barry et al 59 have reported that reducing \(I_{\text{so}}\) by overexpressing an alternative dominant-negative K\(_{\text{v}}\)4.2 polypeptide, which differs from the wild-type protein by only a single point mutation in the pore region (W362F), is not accompanied by obvious hypertrophy or overt heart disease. The discordant phenotype between these 2 models for \(I_{\text{so}}\) reduction might originate from differences in the timing and/or level of transgene expression or differences in genetic backgrounds. Unfortunately, these other investigators did not assess contractile function or \([\text{Ca}^{2+}]\) transients in their transgenic mice to determine whether they also show an

The mechanisms accounting for the progression from the hypercontractile phenotype in young mice to congestive heart failure in the older animals is unclear, although a number of possible explanations exist. A variety of kinases, phosphatases, and receptor signaling pathways contributing to myocyte growth and possibly apoptosis are directly activated by \(\text{Ca}^{2+}\) or use \(\text{Ca}^{2+}\) as an essential cofactor.24,47,48 Thus, the development of heart disease and hypertrophy in these mice might conceivably be linked to sustained elevations in \([\text{Ca}^{2+}]\), secondary to APD prolongation.

Alternatively, the heart failure phenotype in older mice may not be directly linked to APD prolongation and elevated \([\text{Ca}^{2+}]\), but rather to unanticipated and indirect effects of the ectopically expressed mutant K\(_{\text{v}}\)4.2N polypeptide. Recent studies have demonstrated that overexpression of foreign proteins can induce an endoplasmic reticular (ER) stress response, resulting in activation of a number of cell signaling and kinase pathways such as C/EBP homologous protein (CHOP) and the induction of apoptosis.49,50 Consistent with this mechanism, previous studies in cultured myocytes expressing a truncated K\(_{\text{v}}\)4.2-GFP fusion construct 51 as well as GH3 pituitary cells expressing a truncated K\(_{\text{v}}\)1.1 polypeptide 52 have both demonstrated abnormal trapping of the mutant protein in the ER. Indeed, Huang and Izumo 53 have recently described a cardiomyopathy in transgenic mice expressing high levels of a “biologically inert” protein. Thus, it is conceivable that the truncated Kv4.2N polypeptide, which is expressed at levels \(\approx \)10-fold greater than the endogenous full-length protein, may induce a cardiomyopathy by interference with normal cell trafficking and the induction of an ER stress response. Consistent with this alternative mechanism, Barry et al 59 have reported that reducing \(I_{\text{so}}\) by overexpressing an alternative dominant-negative K\(_{\text{v}}\)4.2 polypeptide, which differs from the wild-type protein by only a single point mutation in the pore region (W362F), is not accompanied by obvious hypertrophy or overt heart disease. The discordant phenotype between these 2 models for \(I_{\text{so}}\) reduction might originate from differences in the timing and/or level of transgene expression or differences in genetic backgrounds. Unfortunately, these other investigators did not assess contractile function or \([\text{Ca}^{2+}]\) transients in their transgenic mice to determine whether they also show an

### TABLE 3. Hemodynamic and Echocardiographic Analysis

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<th>Transgenic</th>
<th>Control</th>
<th>Transgenic</th>
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<tr>
<td>Peak aortic pressure, mm Hg</td>
<td>72±2 (20)</td>
<td>85±5 (17)*</td>
<td>91±3 (9)</td>
<td>63±5 (7)*</td>
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<td>Peak LV systolic pressure, mm Hg</td>
<td>73±3 (13)</td>
<td>89±4 (9)*</td>
<td>95±6 (5)</td>
<td>60±5 (6)*</td>
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<td>LV end-diastolic pressure, mm Hg</td>
<td>3.6±0.7 (9)</td>
<td>3.3±0.7 (13)</td>
<td>3.0±0.8 (5)</td>
<td>20.2±2.1 (6)*</td>
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<td>Peak +dP/dt, mm Hg/s</td>
<td>1956±83 (9)</td>
<td>2470±146 (13)*</td>
<td>3458±148 (4)</td>
<td>927±269 (6)*</td>
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<td>Peak −dP/dt, mm Hg/s</td>
<td>−1777±98 (9)</td>
<td>−2284±135 (13)*</td>
<td>−3398±133 (4)</td>
<td>−709±142 (6)*</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>287±11 (17)</td>
<td>267±12 (20)</td>
<td>234±14 (9)</td>
<td>350±19 (7)*</td>
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<td>Fractional shortening</td>
<td>0.45±0.01 (7)</td>
<td>0.50±0.01 (7)*</td>
<td>0.45±0.05 (2)</td>
<td>0.24±0.02 (2)*</td>
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<td>Peak aortic ejection velocity, m/s</td>
<td>0.80±0.022 (6)</td>
<td>0.90±0.04* (6)</td>
<td>0.92±0.03</td>
<td>0.69±0.03*</td>
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Number of individual mice studied is shown in parentheses.

\(*P<0.05\) between age-matched transgenic and control mice.

outward current (\(I_{\text{so}}\)) are among the most common alterations in K\(_{\text{v}}\) currents observed in heart disease.11,13,38–40 In this study, we sought to understand whether alterations of ionic currents such as \(I_{\text{so}}\) in heart disease simply reflect global alterations in cardiac gene expression or whether these electrophysiological changes might contribute to the disease process. Members of the K\(_{\text{v}}\)4.x family have previously been demonstrated to contribute to cardiac \(I_{\text{so}}\) in a number of species and to be downregulated in disease.26,29,41–44 Accordingly, we targeted expression of a dominant-negative K\(_{\text{v}}\)4.2N fragment to the heart using the αMHC promoter, with the expectation that such a strategy would specifically repress cardiac K\(_{\text{v}}\)4.x-dependent currents.30 Generation of stable transgenic lineages harboring the K\(_{\text{v}}\)4.2N transgene was difficult, immediately suggesting that expression of the truncated protein in the heart was poorly tolerated. Although our studies were therefore limited to observations in a single transgenic line, other investigators have reported a similar deleterious phenotype with K\(_{\text{v}}\)4.2 truncation mutants (G. Tomaselli, personal communication, November 1998), suggesting that the observed effect is specific to expression of the mutant protein and not a result of insertional mutagenesis.

Phenotypic evaluation of the MHCα-K\(_{\text{v}}\)4.2N transgenic mice demonstrated progression from a hypercontractile state with normal cardiac morphology to one of profound myocyte hypertrophy, dysfunction, and failure. The enhanced contractility in young transgenic mice is likely a result of prolongation of the APD and the resulting effects on \([\text{Ca}^{2+}]\), transient amplitude. Indeed, \([\text{Ca}^{2+}]\), transients are elevated in transgenic myocytes from 2- to 3.5-week-old mice with prolonged action potentials compared with myocytes with normal action potential profiles (data not shown). Such a relationship between APD prolongation and \([\text{Ca}^{2+}]\), transients has previously been described in dissociated myocytes in several settings, including normal cells subjected to action potential clamp, myocytes from rats with experimental myocardial infarction or with spontaneous hypertension, as well as those transduced with adenoviral vectors expressing K\(_{\text{v}}\)’ channel subunits.21,35,45,46 However, this is the first report, to our knowledge, demonstrating that primary genetic manipulation of the action potential can influence cardiac contractility in vivo.
enhanced contractile state. Clearly, additional studies will be necessary to reconcile these varying phenotypes.

Surface ECGs of anesthetized mice did not disclose convincing evidence of QT prolongation in the Kᵥ4.2N transgenic mice at any stage, despite prolongation of monophasic action potentials in situ and APD prolongation in a significant proportion of isolated myocytes. However, several genetic models in which cardiac repolarizing currents are reduced, including loss of function of minK, and dominant-negative inhibition of ERG, result in no gross perturbations of the surface ECG in anesthetized mice. Given the difficulty of precisely identifying intervals on the mouse surface ECG, the monophasic action potentials may more accurately reflect the time course of repolarization and are certainly consistent with the significant depression of Iᵪ.

Clinically, prolongation of the cardiac action potential has been associated with an increased propensity for cardiac arrhythmias, particularly when heterogeneous in nature. Although death was typically sudden in the Kᵥ4.2N transgenic mice, even in those without obvious signs of congestion, short-term electrocardiographic recordings in anesthetized mice did not reveal the presence of ventricular arrhythmias. We and others have recently used telemetric methodology for long-term electrocardiographic recordings of fully conscious genetically modified mice. Clearly, further studies to systematically determine the propensity for spontaneous or inducible arrhythmogenesis are warranted.
The extent to which our results are applicable to other species is uncertain. Patients with congenital long-QT syndrome rarely show evidence of cardiac hypertrophy, suggesting that the effects of \( I_{\text{K}} \) reduction in our mice might not be applicable to humans. However, all previously identified channel mutations associated with long-QT syndrome affect action potential profiles in a manner that is distinct from changes produced by \( I_{\text{K}} \) reductions and therefore might not produce equivalent effects. Indeed, we have found that prolongation in the early repolarization period has a much greater effect on \([\text{Ca}^{2+}]_i\) than late in repolarization (data not shown). Regardless, to date, no forms of congenital long-QT syndrome have been linked to \( K^+ \) channels producing transient outward currents.\(^{19,20}\)

In summary, we have generated transgenic mice expressing a dominant-negative N-terminal fragment of the \( K_{\text{v}}4.2 \) potassium channel subunit in the heart. Young transgenic mice show heterogeneous reductions in \( I_{\text{K}} \) and APD prolongation, in association with a hypercontractile state. Between 8 and 16 weeks of age, these mice develop a dilated cardiomyopathy with profound cardiac dysfunction, culminating in congestive heart failure and death. Although the mechanism(s) by which ectopic expression of the \( K_{\text{v}}4.2\)N protein in the heart leads to cardiac hypertrophy and failure remains to be clarified, our working hypothesis is that reductions in \( I_{\text{K}} \) and consequent APD prolongation, at least in the rodent, lead to the development of cardiac hypertrophy and heart failure through alterations in calcium delivery and activation of downstream signaling cascades.

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