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# Electrophysiological Properties of Cardiomyocytes Isolated from CYP2J2 Transgenic Mice

MOLECULAR PHARMACOLOGY

Qingen Ke, Yong-Fu Xiao,[1] J. Alyce Bradbury, Joan P. Graves, Laura M. DeGraff, John M. Seubert,[2] and Darryl C. Zeldin

*Cardiovascular Division, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts (Q.K., Y.F.X.); and Division of Intramural Research, National Institute of Environmental Health Sciences/National Institutes of Health, Research Triangle Park, North Carolina (J.A.B., J.P.G., L.M.D., J.M.S., D.C.Z.)*

## ABSTRACT

CYP2J2 is abundant in cardiac tissue and active in the biosynthesis of eicosanoids such as epoxyeicosatrienoic acids (EETs). To determine the effects of CYP2J2 and its eicosanoid products in the heart, we characterized the electrophysiology of single cardiomyocytes isolated from adult transgenic (Tr) mice with cardiac-specific overexpression of CYP2J2. CYP2J2 Tr cardiomyocytes had a shortened action potential. At 90% repolarization, the action potential duration (APD) was $30.6 \pm 3.0$ ms ($n = 22$) in wild-type (Wt) cells and $20.2 \pm 2.3$ ms ($n = 19$) in CYP2J2 Tr cells ($p < 0.005$). This shortening was probably due to enhanced maximal peak transient outward $K^+$ currents ($I_{to,peak}$), which were $38.6 \pm 2.8$ and $54.4 \pm 4.9$ pA/pF in Wt and CYP2J2 Tr cells, respectively ($p < 0.05$). In contrast, the late portion of the transient outward $K^+$ current ($I_{to,280ms}$), the slowly inactivating outward $K^+$ current ($I_{K,slow}$), and the voltage-gated $Na^+$ current ($I_{Na}$) were not significantly altered in CYP2J2 Tr cells. *N*-Methylsulphonyl-6-(2-proparglyloxy-phenyl)hexanamide (MS-PPOH), a specific inhibitor of EET biosynthesis, significantly reduced $I_{to,peak}$ and increased APD in CYP2J2 Tr cardiomyocytes but not in Wt cells. Intracellular dialysis with a monoclonal antibody against CYP2J2 also significantly reduced $I_{to,peak}$ and increased APD in CYP2J2 Tr cardiomyocytes. Addition of 11,12-EET or 8-bromo-cAMP significantly reversed the MS-PPOH- or monoclonal antibody-induced changes in $I_{to,peak}$ and APD in CYP2J2 Tr cells. Together, our data demonstrate that shortening of the action potential in CYP2J2 Tr cardiomyocytes is associated with enhanced $I_{to,peak}$ via an EET-dependent, cAMP-mediated mechanism.

The effects of cytochromes P450 on cardiac function have been extensively studied (Wu et al., 1996, 1997; Zeldin, 2001; Roman, 2002). P450 epoxygenases metabolize arachidonic acid (AA) to *cis*-epoxyeicosatrienoic acids (EETs), which are converted to *vic*-dihydroxyeicosatrienoic acids (DHETs) by epoxide hydrolases (Zeldin, 2001). These P450-derived eicosanoids possess potent biological effects in the cardiovascular system. For example, EETs and DHETs have vasodilatory effects in the coronary circulation (Campbell et al., 1996). Indeed, EETs are leading candidates for endothelium-derived hyperpolarizing factor (Campbell et al., 1996) and may serve as protective agents during cardiac ischemia (Nithipatikom et al., 2001). In isolated guinea pig hearts and single ventricular myocytes, 5,6-EET and 11,12-EET increased cell shortening and intracellular calcium signals (Moffat et al., 1993). The effects of P450 AA metabolites on cardiac ischemia-reperfusion injury have been investigated in several animal models (Granville et al., 2004; Seubert et al., 2004; Nithipatikom et al., 2006; Seubert et al., 2006). A recent canine study showed that 11,12-EET and 14,15-EET had a cardioprotective effect via activation of cardiac $K_{ATP}$ channels (Nithipatikom et al., 2006). Compelling evidence also exists that P450-derived EETs are antihypertensive and anti-inflammatory (Imig, 2005).

Multiple P450s are known to be expressed in cardiac tissue (Wu et al., 1996, 1997; Thum and Borlak, 2000). Among these, CYP2J2 seems to be unique in that it is primarily expressed in the heart, abundant in cardiomyocytes, and

**ABBREVIATIONS:** AA, arachidonic acid; EET, *cis*-epoxyeicosatrienoic acid; DHET, *vic*-dihydroxyeicosatrienoic acid; $\alpha$MHC, $\alpha$-myosin heavy chain; Tr, transgenic; Wt, wild type; MS-PPOH, *N*-methylsulfonyl-6-(2-proparglyloxyphenyl)hexanamide; MAb, monoclonal antibody against; 8-Br-cAMP, 8-bromo-cAMP; APD, action potential duration; PKA, protein kinase A; $I_{to,peak}$, maximal peak transient outward $K^+$ current; $I_{to,280ms}$, late portion of the transient outward $K^+$ current; $I_{Na}$, voltage-gated $Na^+$ current; $K_{ATP}$, ATP-sensitive $K^+$ channel; NS, not significant.

active in EET biosynthesis (Wu et al., 1996, 1997). Seubert et al. (2004) recently showed that transgenic mice with cardiomyocyte-specific overexpression of human CYP2J2 exhibited improved postischemic recovery of left ventricular function. Several mechanisms, including activation of ATP-sensitive $K^+$ ($K_{ATP}$) channels and p42/p44 mitogen-activated protein kinase, enhancement of cardiac cAMP content, shortening of the cardiac action potential and increase in coronary blood flow, might contribute to the improved postischemic functional recovery in CYP2J2 Tr mice. Flavone (2-phenyl-1,4-benzopyrone), which activates P450 metabolic activities, also improved functional recovery after ischemia-reperfusion in rabbit hearts, an effect that was reversed by the P450 inhibitor SKF-525a (Moffat et al., 1993). Wu et al. (1997) found that 11,12-EET improved recovery of contractile function after global ischemia in isolated-perfused rat hearts. Moreover, EETs have been shown to increase cardiomyocyte cAMP content (Xiao et al., 1998), an effect that has been shown to afford cardioprotection after ischemia in canine hearts (Sanada et al., 2001). However, some P450-derived eicosanoids can be detrimental to heart contractile function during postischemic recovery (Moffat et al., 1993; Wu et al., 1997; Gross et al., 2004).

There is evidence that EETs modulate the activities of cardiac ion channels. For example, 8,9-EET inhibits cardiac $Na^+$ channels and shifts the steady-state inactivation to hyperpolarized membrane potentials (Lee et al., 1999). Moffat and coworkers (Moffat et al., 1993) demonstrated that EETs increase intracellular calcium concentrations in isolated guinea pig cardiomyocytes. EETs modulate the activities of cardiac $Ca^{2+}$ channels (Xiao et al., 1998, 2004) and $K_{ATP}$ channels (Lu et al., 2001, 2002). In addition, EETs modulate ion channels in noncardiac cells. Thus, EETs activate $Ca^{2+}$-dependent $K^+$ channels in vascular smooth muscle cells (Li and Campbell, 1997) and modulate transient receptor potential channels (TRPV4) in endothelial cells (Vriens et al., 2005). P450 inhibitors block membrane $Ca^{2+}$ channels in rat thymocytes (Alvarez et al., 1992) and in human neutrophils (Sargeant et al., 1992). EETs enhance L-type $Ca^{2+}$ currents ($I_{Ca}$) in rat cardiomyocytes via increase in intracellular cAMP content (Xiao et al., 1998). EETs also have been reported to inhibit cardiac $Na^+$ channels (Lee et al., 1999). Therefore, ion channels may constitute one of the major effectors of EET actions.

We have used the cardiomyocyte-specific $\alpha$-myosin heavy chain ($\alpha$MHC) promoter to overexpress human CYP2J2 in transgenic mice (Seubert et al., 2004). Hearts from CYP2J2 transgenic (Tr) mice have increased cardiac CYP2J2 mRNA and protein expression and increased cardiomyocyte AA epoxygenase activity compared with wild-type (Wt) mice (Seubert et al., 2004). Cardiac L-type $Ca^{2+}$ currents and $K_{ATP}$ currents were significantly enhanced in this transgenic model (Xiao et al., 2004; Lu et al., 2006). Moreover, CYP2J2 Tr hearts have improved postischemic recovery of left ventricular function (Seubert et al., 2004). In the current study, we examined the properties of action potentials and other ion channels in cardiomyocytes isolated from these transgenic mice. Our data show that the duration of action potentials are significantly shortened in CYP2J2 Tr heart cells and that this shortening is associated with an enhancement of $I_{to,peak}$ via an EET-dependent, cAMP-mediated mechanism.

## Materials and Methods

**Materials.** *N*-Methylsulfonyl-6-(2-propargyloxyphenyl)hexanamide (MS-PPOH), a specific inhibitor of EET biosynthesis, was synthesized as described previously (Wang et al., 1998). Working stocks of MS-PPOH (50 mM) were prepared in 100% ethanol and stored under argon at $-20°C$. 11,12-EET was prepared by total chemical synthesis and purified by reverse-phase HPLC as described previously (Wu et al., 1997; Chen et al., 1999; Node et al., 2001). The monoclonal antibody (MAb-1, Lot 6-2-16-1) against the recombinant CYP2J2 protein was generated in mouse hybridoma cells as described previously (Gelboin et al., 1998; Krausz et al., 2000; Xiao et al., 2004). The antibody was diluted by the internal pipette solution to a final concentration of 0.125 mg/ml IgG for intracellular dialysis (see below). A monoclonal antibody (MAb, lot 12-10-96) against egg lysozyme served as a negative control. 8-Br-cAMP was obtained from Sigma (St. Louis, MO). Polyclonal rabbit anti-KChIP2 was purchased from Affinity Bioreagents, Inc. (Golden, CO). Polyclonal rabbit anti-Kv4.2 and anti-Kv1.4 were purchased from Exalpha Biologicals, Inc. (Maynard, MA). Polyclonal rabbit anti-Kv4.3 was purchased from BioSource International, Inc. (Camarillo, CA). Mouse monoclonal antibody raised against purified rabbit $Na^+/K^+$-ATPase $\alpha 1$ was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

**Generation of CYP2J2 Tr Mice.** The coding region of the CYP2J2 cDNA (GenBank U37143) was cloned into the SalI-HindIII sites of the vector pBS-$\alpha$MHC-hGH (clone 26), a generous gift from Dr. Jeffrey Robbins (University of Cincinnati). This vector contains the $\alpha$MHC promoter to drive cardiomyocyte-specific expression of the CYP2J2 transgene and human growth hormone intron/polyA sequences to enhance transgene mRNA stability (Seubert et al., 2004). The plasmid was digested with NotI, and the linearized transgene was microinjected into pronuclei of single cell C57BL6/J mouse embryos that were implanted into pseudopregnant female mice. Founder pups were identified by a combination of polymerase chain reaction and Southern blotting of tail genomic DNAs as described previously (Seubert et al., 2004). All studies used heterozygous CYP2J2 Tr mice and age/sex-matched Wt littermate control mice. All studies were in accordance with principles outlined in the *NIH Guide for the Care and Use of Laboratory Animals* and were approved by the Animal Care and Use Committees at the respective institutions.

**Isolation of Single Ventricular Cardiomyocytes.** Single left ventricular myocytes were enzymatically isolated from CYP2J2 Tr and Wt hearts using methods described previously (Xiao et al., 1998). In brief, hearts were rapidly excised, cannulated via the aorta, and connected to a modified Langendorff apparatus. Hearts were initially perfused for 4 min at a flow rate of $\sim$3 ml/min with oxygenated 37°C Tyrode's solution containing 137 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 10 mM glucose, and 10 mM HEPES, pH 7.4. Hearts were then perfused with $Ca^{2+}$-free Tyrode's solution for 5 to 6 min, recirculated with $Ca^{2+}$-free Tyrode's solution containing 0.7 mg/ml collagenase (type I) and 0.02 mg/ml protease (type XIV) (Sigma-Aldrich) for 10–15 min, and finally perfused with Tyrode's solution containing 200 $\mu$M $CaCl_2$ for 5 min. Several pieces of myocardium were then removed from the left ventricle, placed into a Petri dish with Tyrode's solution containing 200 $\mu$M $CaCl_2$, minced and gently agitated to separate the cells, and maintained at room temperature for up to 2 h. Quiescent, rod-shaped ventricular myocytes with clear striations were randomly selected for electrophysiology studies.

**Electrophysiological Recordings.** Action potentials were measured under current-clamp conditions, and $K^+$ and $Na^+$ currents were measured under voltage-clamp conditions with the whole-cell patch-clamp configuration at room temperature (22–24°C) as described previously (Xiao et al., 1998, 2004). In brief, glass electrodes (World Precision Instruments, Sarasota, FL) with 1- to 2-M$\Omega$ resistance were connected via a Ag-AgCl wire to an Axopatch 1D amplifier interfaced to a DigiData 1320 data acquisition system controlled

by pCLAMP software 8.02 (Molecular Devices, Sunnyvale, CA). After forming a conventional gigaohm seal between the recording electrode and the myocyte membrane, electrode capacitance was fully compensated. Additional suction was used to form the whole-cell configuration. The membrane capacitance (measured with pClamp software, version 8.2) was $122.4 \pm 3.5$ pF for Wt cardiomyocytes ($n = 81$) and $119.3 \pm 3.2$ pF for the CYP2J2 Tr cardiomyocytes ($n = 96$, p = NS). After the capacitance measurement, whole-cell membrane capacitance and series resistance were electrically compensated by ~90% to reduce artifactual distortion. For action potential recordings, myocytes were superfused at a rate of 2 to 3 ml/min with the Tyrode's solution containing 2 mM $CaCl_2$. The pipette solution consisted of 90 mM potassium aspartate, 40 mM KCl, 1 mM $MgCl_2$, 3 mM Mg-ATP, 10 mM EGTA, and 10 mM HEPES, pH 7.3. After forming the whole-cell configuration, the experimental protocols began immediately to collect initial data. The holding potential was set to approximately $-75$ mV. For the whole-cell recording of $K^+$ currents, the bath solution contained 137 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 0.1 mM $CdCl_2$, 0.02 mM tetrodotoxin, 10 mM glucose, and 10 mM HEPES, pH 7.4, and the pipette solution contained 50 mM KCl, 80 mM potassium aspartate, 1 mM $MgCl_2$, 10 mM EGTA, 3 mM Mg-ATP, and 10 mM HEPES, pH 7.2. For the whole-cell recording of $Na^+$ currents, the bath solution contained 120 mM $N$-methyl $d$-glucamine, 20 mM NaCl, 5 mM CsCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 0.1 mM $CdCl_2$, 10 mM glucose, and 10 mM HEPES, pH 7.4, and the pipette solution contained 100 mM CsCl, 40 mM CsOH, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 11 mM EGTA, 5 mM Mg-ATP, and 10 mM HEPES, pH 7.3. For data acquisition, filter parameters were at 2 kHz and sampling rates were at 3 to 5 kHz.

**Protein Immunoblotting.** Lysates were prepared from frozen mouse hearts as described previously (Wu et al., 1997). Immunoblotting with rabbit anti-KChIP2 (2 $\mu$g/ml), rabbit anti-Kv4.2 (2 $\mu$g/ml), rabbit anti-Kv1.4 (5 $\mu$g/ml), rabbit anti-Kv4.3 (2 $\mu$g/ml), and mouse anti-$Na^+/K^+$-ATPase $\alpha1$ (1:200 dilution) was performed according to the manufacturers' instructions.

**Statistical Analysis.** The density (picoamperes per picofarad) of ion current was calculated as a ratio of current amplitude to membrane capacitance of individual cardiomyocytes to avoid the possibility that the differences in ion currents in CYP2J2 Tr and Wt cardiomyocytes resulted from differences in cell size. Inactivation time constants were determined by least-squares fitting to each current traces (Xiao et al., 1998, 2004). The results of the steady-state inactivation of $I_{Na}$ were fitted by a Boltzmann equation ($y = 1/\{1 + \exp[(V - V_{0.5})/K]\}$). The best-fit procedure was performed with a commercial software program (Origin 7.0; OriginLab Corp., Northampton, MA). All data are presented as mean $\pm$ S.E.M. unless otherwise stated. Paired or unpaired Student's $t$ test or one way analysis of variance (ANOVA) was applied for statistical analyses as appropriate. Differences were considered significant if $p < 0.05$.

## Results

We have shown previously that CYP2J2 Tr hearts have increased CYP2J2 expression and an increased capacity to metabolize AA to EETs compared with Wt hearts (Seubert et al., 2004). Moreover, isolated cardiomyocytes from CYP2J2 Tr mice release significantly more stable EET metabolites into their culture media than do cardiomyocytes from Wt mice (Seubert et al., 2004). Together, these data are consistent with overexpression of a catalytically active transgene in the hearts of CYP2J2 Tr mice.

**Cardiac Action Potential.** Cardiomyocyte $Na^+$, $Ca^{2+}$, and $K^+$ channels have all been shown to be modulated by EETs (Xiao et al., 1998; Lee et al., 1999; Lu et al., 2001, 2006). To investigate the net effect of CYP2J2-derived EETs on cardiac electrophysiology, we first examined the cardiac

action potential in the CYP2J2 Tr mice and Wt control mice. The resting membrane potential was $-62.2 \pm 1.5$ mV in Wt cardiomyocytes ($n = 41$) and $-62.6 \pm 1.7$ mV in CYP2J2 Tr cardiomyocytes ($n = 35$, $p = $ NS). There were also no significant differences in action potential amplitude, action potential threshold, or the maximum upstroke velocity between the two groups (Fig. 1a, Table 1). However, action potential duration (APD) was significantly shorter in CYP2J2 Tr versus Wt cardiomyocytes at both 50 and 90% repolarization ($p < 0.005$) (Fig. 1a, Table 1).

**Cardiac Outward $K^+$ Currents and Voltage-Gated $Na^+$ Currents.** Shortening of the cardiac action potential can be due to increased outward $K^+$ currents (Xu et al., 1999; Nerbonne, 2000); hence, we examined outward $K^+$ currents in CYP2J2 Tr and Wt cardiomyocytes using the whole-cell, voltage-clamp method. Depolarizing steps produced outward currents that rose rapidly to a peak and then decayed (Fig. 1, b–d). Maximal peak transient outward $K^+$ currents ($I_{to,peak}$) were significantly increased in CYP2J2 Tr cardiomyocytes relative to Wt cardiomyocytes ($p < 0.01$) (Fig. 1, b and c, Table 2). In contrast, there were no significant differences between the two groups in the late portion of the transient outward $K^+$ current measured at 280 ms ($I_{to,280ms}$) or in the slowly inactivating $K^+$ current ($I_{K,slow}$) (Fig. 1d, Table 2). Potassium currents elicited by 5-s voltage pulses from a holding potential of $-60$ to $+40$ mV fitted well by the sum of two exponential decay. The fast time constant ($\tau_{fast}$) was $75.4 \pm 3.9$ ms for Wt ($n = 18$) and $47.5 \pm 2.1$ ms for CYP2J2 Tr ($n = 24$) cells ($p < 0.001$). The slow time constant ($\tau_{slow}$) was $1195 \pm 58$ ms for Wt and $1233 \pm 82$ ms for CYP2J2 Tr cells ($p = $ NS). Thus, the faster decay in the CYP2J2 Tr cells resulted from larger peak currents with similar amplitude of late currents. Changes in time constants for other voltage pulses paralleled the above parameters (data not shown). We also analyzed the other components of the outward $K^+$ currents including $I_{Kr}$ and $I_{Ks}$ but found no significant differences between Wt and CYP2J2 Tr cardiomyocytes. Maximal inward $K^+$ currents elicited by hyperpolarizing pulses ($I_{K1}$) were similar in CYP2J2 Tr and Wt cardiomyocytes (Table 2).

Because the voltage-gated $Na^+$ current ($I_{Na}$) could also affect cardiac APD, we compared the properties of $I_{Na}$ in cardiomyocytes isolated from Wt and CYP2J2 Tr mice to determine whether cardiac overexpression of CYP2J2 altered the function of cardiac $Na^+$ channels. Figure 2, a–c, shows that whole-cell currents recorded from single left ventricular myocytes were very similar in amplitude, activation, inactivation, and shape of the current-voltage relationship curves between Wt and CYP2J2 Tr mice. In addition, the normalized steady-state inactivation of cardiac $Na^+$ currents was also very similar among the cardiomyocytes isolated from the Wt and CYP2J2 Tr mice (Fig. 2d). The $V_{0.5}$ of the steady-state inactivation was $-87.7 \pm 0.7$ mV with a slope of 7.3 for Wt cardiomyocytes and $-88.6 \pm 0.6$ mV with a slope factor of 5.7 for CYP2J2 Tr cardiomyocytes, respectively. These results suggest that cardiac overexpression of CYP2J2 did not significantly alter the function of the voltage-gated $Na^+$ channels.

**Effects of an EET Biosynthesis Inhibitor on $I_{to,peak}$ and APD.** To determine whether P450 activity affected cardiac outward $K^+$ currents in CYP2J2 Tr cardiomyocytes, we added MS-PPOH to the external bath solution and then elicited outward $K^+$ currents by 5-s depolarizing pulses from

−50 to 70 mV in 10-mV increments with a holding potential of −60 mV (Fig. 3). Extracellular application of 25 $\mu$M MS-PPOH did not significantly affect the peak amplitude and

inactivation time constant of outward K$^+$ currents in Wt cardiomyocytes (Fig. 3a, Table 3). However, MS-PPOH significantly reduced the peak amplitude of outward K$^+$ cur-
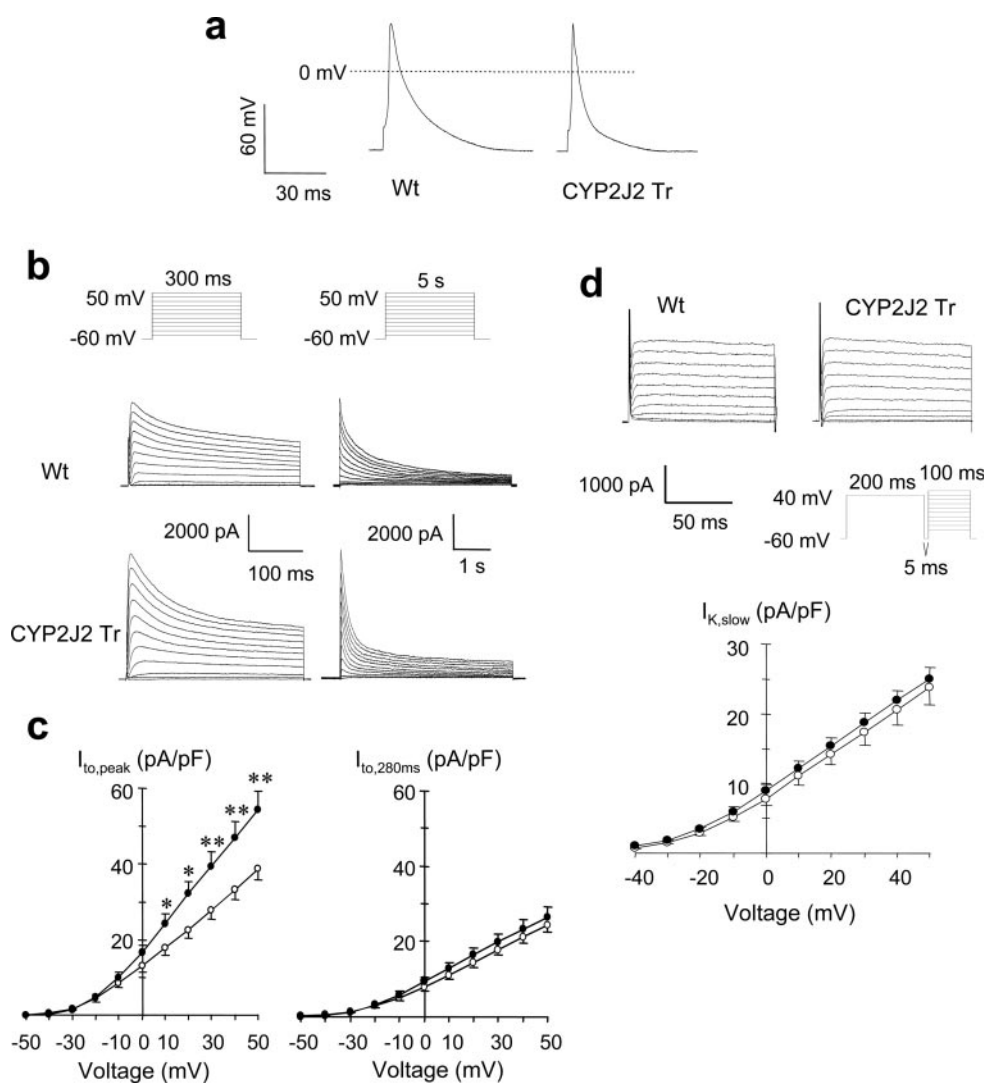
**Fig. 1.** Characterization of action potentials and outward K$^+$ currents in the CYP2J2 Tr and Wt cardiomyocytes. a, representative action potentials recorded from ventricular myocytes isolated from Wt and CYP2J2 Tr hearts. The action potentials were elicited by intracellular injection of depolarizing current pulses (15 pA with 10 ms duration) from a holding membrane potential of approximately −75 mV. b, superimposed original traces of transient outward K$^+$ currents recorded from two representative cardiomyocytes isolated from Wt and CYP2J2 Tr mice. Whole-cell outward K$^+$ currents were evoked by 300 ms (left) or 5 s (right) depolarizing pulses from −50 to +50 mV with 10-mV increments every 10 s. The membrane holding potential was set at −60 mV. c, current-voltage relationship of outward K$^+$ currents recorded from single ventricular myocytes. Left, the peak amplitude of transient outward K$^+$ currents (I$_{to,peak}$) was measured and averaged from individual cardiomyocytes (○, Wt, $n$ = 22; ●, CYP2J2 Tr, $n$ = 23). Right, the late portion of transient outward K$^+$ currents was measured at the point of 280 ms (I$_{to,280ms}$) and averaged from individual cardiomyocytes (○, Wt, $n$ = 22; ●, CYP2J2 Tr, $n$ = 23). The current density was calculated by the amplitude of current divided by the membrane capacitance of each cell. *, $p < 0.05$ versus Wt; **, $p < 0.01$ versus Wt. d, slowly inactivating outward K$^+$ currents (I$_{K,slow}$) recorded from isolated single ventricular myocytes. Top, current traces from representative Wt and CYP2J2 Tr cells elicited by test pulses from −40 to 50 mV with 10-mV increments every 10 s. A prepulse of 200 ms from the holding potential of −60 to +40 mV was applied and followed a 5-ms recovery interval to −60 mV. The protocol minimized contamination of transient outward current (I$_{to}$), the fast inactivating component of the depolarization-activated K$^+$ currents. Bottom, the current-voltage relationships of I$_{K,slow}$ for Wt (○, $n$ = 18) and CYP2J2 Tr (●, $n$ = 24) cardiomyocytes.

TABLE 1
Action potential characteristics of ventricular myocytes isolated from CYP2J2 Tr mice and Wt control mice
Values are means ± S.E.

|  | Mouse | Cell | APA | APT | $V_{max}$ | APD$_{50}$ | APD$_{90}$ |
|---|---|---|---|---|---|---|---|
|  | $n$ | $n$ | $mV$ | $pA$ | $V/s$ | $ms$ | $ms$ |
| Wt | 11 | 22 | 116.9 ± 5.1 | 22.9 ± 1.9 | 44.2 ± 4.9 | 8.65 ± 0.86 | 30.6 ± 3.0 |
| CYP2J2 Tr | 9 | 19 | 122.1 ± 6.2 | 21.1 ± 2.2 | 49.6 ± 6.3 | 5.86 ± 0.57* | 20.2 ± 2.4* |

APA, action potential amplitude; APT, action potential threshold (minimal depolarizing currents required for initiation of the first action potential); $V_{max}$, maximum upstroke velocity; APD, action potential duration measured at 50% (APD$_{50}$) and 90% (APD$_{90}$) repolarization.
* $p < 0.005$ versus Wt.

rents ($I_{to,peak}$) in CYP2J2 Tr cardiomyocytes to 70% of the pretreated level ($n = 12$, $p < 0.01$) (Fig. 3b, Table 3). The MS-PPOH inhibitory effect on $I_{to,peak}$ was observed after 5 to 10 min of perfusion and was reversed during washout (Fig. 3c). It is noteworthy that addition of 2 mM membrane permeable 8-Br-cAMP to the bath solution restored the MS-PPOH-inhibited currents to 95% of the pretreated level (Fig. 3b, Table 3). In addition, MS-PPOH significantly slowed the fast inactivation time constant ($\tau_{fast}$) of outward $K^+$ currents in CYP2J2 Tr cardiomyocytes from $47.7 \pm 2.0$ to $68.5 \pm 3.3$ ms ($n = 12$, $p < 0.01$) (Table 3). The fast inactivation time constant was returned to the pretreated level after extracellular perfusion of 2 mM 8-Br-cAMP (Table 3). Together, these results indicate that 1) inhibition of EET biosynthesis in CYP2J2 Tr cardiomyocytes significantly reduced $I_{to,peak}$ and slowed the fast inactivation of the outward $K^+$ current and 2) treatment with cAMP attenuated these effects.

We also assessed the effects of MS-PPOH on action potentials in Wt and CYP2J2 Tr cardiomyocytes. MS-PPOH significantly prolonged the duration of action potentials in CYP2J2 Tr cardiomyocytes. Thus, the duration of action potentials at 90% repolarization was prolonged from $20.5 \pm 2.1$ ms (initial value) to $30.1 \pm 3.0$ ms ($n = 10$, $p < 0.05$) (Table 4) in CYP2J2 Tr cardiomyocytes. In contrast, MS-PPOH did not significantly prolong APD in Wt cardiomyocytes (Table 4). It is noteworthy that extracellular application of the membrane permeable 8-Br-cAMP (2 mM) restored the duration of action potentials to near initial levels in CYP2J2 Tr cardiomyocytes treated with MS-PPOH (Table 4). Together, these results indicate that inhibition of EET biosynthesis in CYP2J2 Tr cardiomyocytes significantly prolonged the action potential and that treatment with cAMP attenuated these effects.

**Effects of a CYP2J2 Inhibitory Monoclonal Antibody on $I_{to,peak}$ and APD.** An inhibitory monoclonal antibody against CYP2J2 (MAb-1) was previously developed to facilitate studies on the role of this P450 in cellular electrophysiology (Xiao et al., 2004). MAb-1 strongly inhibits activity of recombinant CYP2J2 but does not inhibit activity of nonCYP2J subfamily P450s, including members of the CYP1A, CYP1B, CYP2A, CYP2B, CYP2C, CYP2D, CYP2E, CYP3A, and CYP4A subfamilies (Xiao et al., 2004). To assess whether the enhanced cardiac $I_{to,peak}$ in CYP2J2 Tr mice were related to overexpression of CYP2J2, we internally dialyzed the MAb-1 in cardiomyocytes to selectively inhibit CYP2J2 activity. The amplitude of $I_{to,peak}$ measured immediately after rupturing the membrane and forming the
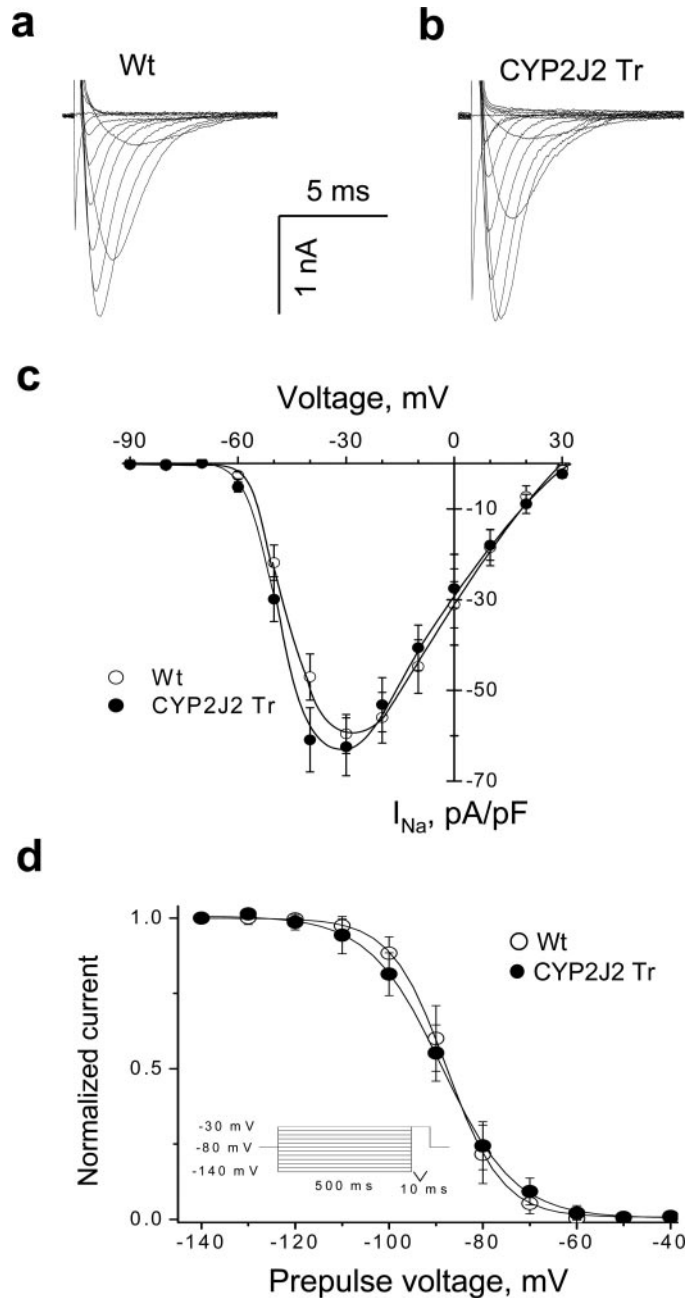


**Fig. 2.** Comparison of voltage-gated $Na^+$ currents in cardiomyocytes isolated from Wt and CYP2J2 Tr mice. a and b are superimposed whole-cell current traces recorded from single left ventricular cardiomyocytes isolated from Wt (a) and CYP2J2 Tr (b) mouse hearts. The currents were elicited by voltage pulses (10 ms duration every 5 s) from a holding potential of $-80$ mV down to $-90$ mV and up to 30 mV with 10-mV increments. c, current-voltage relationship curves for Wt ($\bigcirc$, $n = 15$) and CYP2J2 Tr ($\bullet$, $n = 11$) cardiomyocytes are shown. d, normalized steady-state inactivation of cardiac $Na^+$ currents. Currents were elicited by a double-pulse protocol (inset) composed of a 10-ms test pulse to $-30$ mV after a 500 ms conditioning prepulse varying from $-140$ to $-40$ mV with 10-mV increments every 10 s from a holding potential of $-80$ mV. Peak $Na^+$ currents elicited by test pulses were normalized to the maximal currents recorded with the prepulses from $-140$ to $-40$ mV and plotted against the prepulse voltages for the Wt ($\bigcirc$) and CYP2J2 Tr ($\bullet$) cardiomyocytes. The inactivation data points of peak $I_{Na}$ were fitted to a Boltzmann equation.

TABLE 2

Potassium currents recorded from cardiomyocytes isolated from CYP2J2 Tr mice and Wt control mice

Current density (picoamperes per picofarad) was calculated by the amplitude of currents divided by the membrane capacitance of individual cardiomyocytes. $I_{to, peak}$ is the maximal peak transient outward $K^+$ currents; $I_{to,280 ms}$ is the transient outward $K^+$ current measured at the point of $_{280 ms}$. $I_{to}$ was elicited by a 300-ms test pulse from a holding potential of $-60$ to $+50$ mV every 10 s. $I_{K, slow}$, a slowly inactivating outward $K^+$ current was measured as the maximal current during a 100-ms test pulse from $-60$ to $+50$ mV after a 200-ms conditioning pulse from $-50$ to $+40$ mV and a 5-ms interval at $-50$ mV (see the protocol of Figure 3D). $I_{K1}$ was measured as the maximal inward currents elicited by 300-ms hyperpolarizing pulses from a holding potential of $-40$ to $-100$ mV. All values are mean $\pm$ S.E.M. The peak current density data under control conditions and after MS-PPOH and cAMP treatments were reported elsewhere in preliminary form (Xiao, 2007).

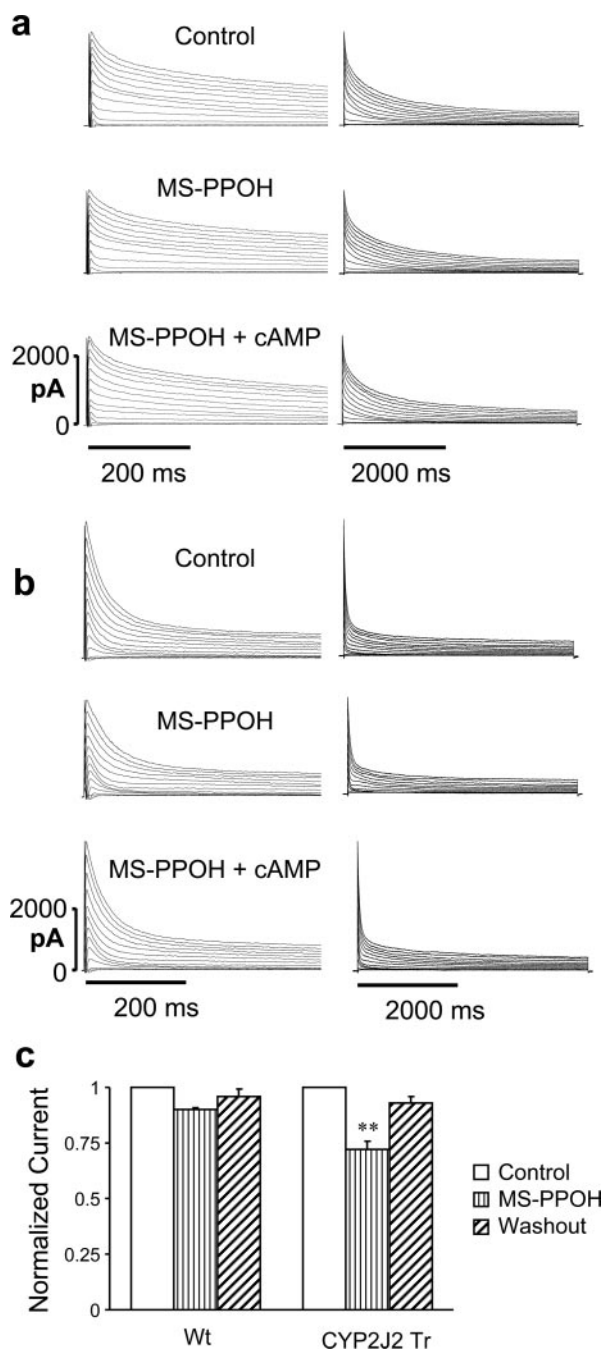|  | $I_{to, peak}$ | $I_{to,280 ms}$ | $I_{K, slow}$ | $I_{K1}$ |
|---|---|---|---|---|
| Wt | $38.6 \pm 2.8$ | $24.3 \pm 1.8$ | $23.8 \pm 1.6$ | $-19.2 \pm 2.4$ |
|  | ($n = 22$) | ($n = 22$) | ($n = 18$) | ($n = 14$) |
| CYP2J2 Tr | $54.4 \pm 4.9*$ | $26.3 \pm 2.9$ | $25.0 \pm 2.5$ | $-18.3 \pm 2.0$ |
|  | ($n = 23$) | ($n = 23$) | ($n = 24$) | ($n = 12$) |

* $p < 0.01$ versus Wt.

**Fig. 3.** Suppression of $I_{to,peak}$ by the P450 epoxygenase inhibitor MS-PPOH in CYP2J2 Tr cardiomyocytes. Whole-cell outward $K^+$ currents were evoked by 5-s (right panels) depolarizing pulses from $-50$ mV to 70 mV in 10 mV increments. The membrane holding potential was $-60$ mV, and the pulse rate was 0.1 Hz. The early portions (600 ms) of the currents are shown in the corresponding left panels (see the protocols in Fig. 1b). a, the effect of extracellular application of 25 $\mu$M MS-PPOH on outward $K^+$ currents in a Wt cardiomyocyte. Addition of 2 mM 8-Br-cAMP after MS-PPOH application did not significantly alter the outward $K^+$ currents. b, bath perfusion with 25 $\mu$M MS-PPOH significantly inhibited $I_{to,peak}$ but did not affect the late portion of $I_{to}$ ($I_{to,280ms}$) or the delayed outward $K^+$ current ($I_{k,slow}$) in a CYP2J2 Tr cardiomyocyte. Addition of 2 mM 8-Br-cAMP reversed the MS-PPOH-induced inhibition of $I_{to,peak}$. c, averaged data from multiple experiments showing inhibition of $I_{to,peak}$ by 25 $\mu$M MS-PPOH in CYP2J2 Tr cardiomyocytes and reversal of this effect after washout. Currents were evoked by 5-s single-step pulses from $-60$ to 70 mV. The pulse rate was 0.1 Hz with a holding potential of $-60$ mV. The maximal peak amplitude of $I_{to}$ was measured. Data are presented as mean $\pm$ S.E. **, $p < 0.01$ versus Wt.

whole-cell configuration was taken as the control value. $I_{to,peak}$ gradually decreased after intracellular dialysis with MAb-1 at a concentration of 0.125 mg/ml IgG. At 15 min after initiation of dialysis, $I_{to,peak}$ was suppressed in both Wt and CYP2J2 Tr cardiomyocytes (Figs. 4 and 5); however, the inhibition of $I_{to,peak}$ reached statistical significance only in CYP2J2 Tr cardiomyocytes (Fig. 4c, Table 3). Thus, $I_{to,peak}$ was reduced by 14.0 $\pm$ 4.2% ($n = 9$, p = NS) after dialysis with MAb-1 in Wt cardiomyocytes, whereas the corresponding reduction of $I_{to,peak}$ was 38.0 $\pm$ 5.9% ($n = 7$, $p < 0.05$) in CYP2J2 Tr cardiomyocytes. By comparison, a control MAb prepared against egg lysozyme had no significant effects on $I_{to,peak}$.

The inhibition of $I_{to,peak}$ after intracellular dialysis with MAb-1 in CYP2J2 Tr cardiomyocytes developed gradually and usually took 8 to 12 min to reach a new, lower steady-state level. It is noteworthy that bath perfusion of either 11,12-EET (40 nM) or the membrane permeable 8-Br-cAMP (2 mM) almost completely reversed the inhibition of $I_{to,peak}$ in CYP2J2 Tr cardiomyocytes dialyzed with MAb-1 (Figs. 4 and 5, Table 3). Together, these results indicate that selective inhibition of CYP2J2 activity with MAb-1 results in a significant reduction of cardiomyocyte $I_{to,peak}$ and that either EET or cAMP can restore the inhibited currents.

We also assessed the effects of intracellular dialysis with MAb-1 on action potentials in Wt and CYP2J2 Tr cardiomyocytes. Intracellular dialysis with the MAb-1 at 0.125 mg/ml IgG significantly prolonged the duration of action potentials in CYP2J2 Tr cardiomyocytes (Fig. 6, bottom), but did not significantly prolong APD in Wt cardiomyocytes (Fig. 6, top). At 15 min after initiation of dialysis, APD at 90% repolarization was prolonged from 21.5 $\pm$ 2.5 ms (initial value) to 30.4 $\pm$ 2.8 ms ($n = 9$, $p < 0.01$) (Table 4). Significant prolongation was not observed in Wt cardiomyocytes dialyzed with the MAb-1 (Table 4). It is noteworthy that extracellular application of either 11,12-EET (40 nM) (Fig. 6) or the membrane permeable 8-Br-cAMP (2 mM) restored the duration of action potentials to near initial levels in CYP2J2 Tr cardiomyocytes dialyzed with MAb-1 (Table 4). Together, these results demonstrate that the shortened APD in CYP2J2 Tr cardiomyocytes is due to EET-mediated effects via a cAMP-dependent mechanism.

**Expression of Voltage-Gated $K^+$ Channels.** To determine whether the increase in $I_{to,peak}$ in CYP2J2 Tr cardiomyocytes could be due to up-regulation of voltage-gated $K^+$ channels, we examined expression of Kv1.4, Kv4.2, Kv4.3, and KChIP2 by immunoblotting. As shown in Fig. 7A, the antibody to KChIP2 detected two prominent bands (33 and 26 kDa) in heart lysates from Wt and CYP2J2 Tr mice. The antibody to Kv1.4 detected a single 73-kDa band in heart lysates from Wt and CYP2J2 Tr mice. The antibody to Kv4.2 detected a 70-kDa band in heart lysates from Wt and CYP2J2 Tr mice. In each case, there were no significant differences in the abundance of these protein bands between the two genotypes after normalization to expression of the control protein $Na^+/K^+$-ATPase $\alpha$1 (Fig. 7B). The antibody to Kv4.3 did not detect any bands in the expected molecular mass range (89 kDa) in heart lysates from either Wt or CYP2J2 Tr mice. Based on this data, we conclude that the expression of outward $K^+$ channels is not significantly different in CYP2J2 Tr hearts.

## Discussion

In the present study we have shown that overexpression of CYP2J2 in cardiomyocytes significantly shortened the duration of action potentials. Such shortening was probably caused by enhanced maximal $I_{to,peak}$ because overexpression of CYP2J2 did not significantly alter the function of the voltage-gated $Na^+$ channels. There has been considerable evidence that cardiac P450s can affect heart function. CYP2J2 is abundant in the heart and its expression is highly localized to cardiomyocytes (Wu et al., 1996, 1997). This P450 epoxygenase is the major cardiac enzyme responsible for generating the EETs, biologically active eicosanoids (Wu et al., 1996). Human and rodent hearts contain substantial quantities of EETs, which have been shown to influence cardiac function (Wu et al., 1996, 1997; Zeldin, 2001; Roman, 2002). For example, the EETs are potent coronary artery vasodilators (Campbell et al., 1996) and are known to affect cardiac ATP-sensitive $K^+$ channels (Lee et al., 1999; Lu et al., 2001, 2002). Studies on the biological effects of P450 metabolites in the heart have often produced conflicting results. For example, EETs are reported to both stimulate (Xiao et al., 1998) and inhibit (Chen et al., 1999) cardiac L-type $Ca^{2+}$ channels, and have been shown to have both positive (Moffat et al., 1993; Xiao et al., 1998) and negative (Lu et al., 2001) inotropic effects in the heart under basal conditions. After ischemia-reperfusion, EETs are reported to have both cardio-protective (Nithipatikom et al., 2001, 2006; Seubert et al.,

2006, 2007) and cardiodepressant (Moffat et al., 1993) effects. In light of these controversies, we developed a transgenic murine model to study the effects of CYP2J2 overexpression on cardiac function. Our previous work has shown that CYP2J2 Tr hearts have enhanced EET biosynthesis and improved postischemic recovery of left ventricular function (Seubert et al., 2004). We have also found that CYP2J2 Tr cardiomyocytes have enhanced $I_{Ca}$ via a mechanism that involves cAMP-protein kinase A-dependent phosphorylation of the L-type $Ca^{2+}$ channel (Xiao et al., 2004). Our current results demonstrate that overexpression of CYP2J2 in cardiomyocytes also causes shortening of the action potential and increases outward $K^+$ currents ($I_{to,peak}$).

Inhibition of P450 activity by MS-PPOH or the specific CYP2J2 monoclonal antibody MAb-1 significantly increased the duration of action potentials and decreased $I_{to,peak}$ in the CYP2J2 Tr cardiomyocytes, but not in Wt cells. It has been shown previously that MS-PPOH is a potent and selective inhibitor of P450-catalyzed AA epoxidation in vitro and in vivo (Wang et al., 1998; Brand-Schieber et al., 2000). It is noteworthy that application of the CYP2J2 metabolite 11,12-EET significantly reversed the MAb-1 or MS-PPOH-induced effects on APD and $I_{to,peak}$. Based on this data, we conclude that MAb-1 or MS-PPOH-induced alterations in APD and $I_{to,peak}$ are probably due to inhibition of P450 AA epoxygenase activity. Our data also suggest that the effect of CYP2J2 overexpression on $I_{to,peak}$ is mediated by P450-derived me-

**TABLE 3**
Effects of MS-PPOH and MAb-1 on peak current density and inactivation time constant
The peak current density data under control conditions and after MS-PPOH and cAMP treatments were reported elsewhere in preliminary form (Xiao, 2007).

| | Wt | | | | | | CYP2J2 Tr | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Peak | | | Inactivation ($\tau_{fast}$) | | | Peak | | | Inactivation ($\tau_{fast}$) | | |
| | $n$ | Mean | SE | $n$ | Mean | SE | $n$ | Mean | SE | $n$ | Mean | SE |
| | *pA/pF* | | | *ms* | | | *pA/pF* | | | *ms* | | |
| Control | 11 | 36.7 | 3.6 | 11 | 74.4 | 3.8 | 12 | 56.3 | 3.8 | 12 | 47.7 | 2.0 |
| MS-PPOH | 11 | 32.5 | 2.5 | 11 | 74.8 | 3.0 | 12 | 39.5** | 2.2 | 12 | 68.5** | 3.1 |
| cAMP | 11 | 33.6 | 3.3 | 11 | 72.7 | 4.2 | 12 | 53.4 | 3.9 | 12 | 49.2 | 2.2 |
| Initial | 9 | 38.8 | 2.6 | 9 | 80.2 | 3.5 | 7 | 57.2 | 3.1 | 7 | 49.0 | 3.1 |
| MAb-1 | 9 | 32.9 | 3.0 | 9 | 78.5 | 5.2 | 7 | 40.8** | 2.5 | 7 | 70.4** | 3.8 |
| cAMP | 9 | 35.2 | 3.0 | 9 | 77.1 | 6.1 | 7 | 52.1 | 3.3 | 7 | 66.6 | 3.8 |
| Initial | 9 | 40.0 | 2.9 | 9 | 77.8 | 4.3 | 11 | 55.8 | 2.7 | 11 | 46.3 | 2.5 |
| MAb-1 | 9 | 35.1 | 3.4 | 9 | 77.0 | 5.2 | 11 | 39.6** | 2.3 | 11 | 71.0** | 3.3 |
| EET | 9 | 39.8 | 3.5 | 9 | 73.2 | 5.8 | 11 | 54.9 | 3.0 | 11 | 46.1 | 2.9 |

Currents were elicited the 5-s test pulses from holding potential of $-60$ to $+50$ mV every 15 s.
** $p < 0.01$

**TABLE 4**
Effects of MS-PPOH and MAb-1 on the action potential duration
Values are the means $\pm$ S.E. cAMP and EET were given after application of MS-PPOH and after dialysis with MAb-1.

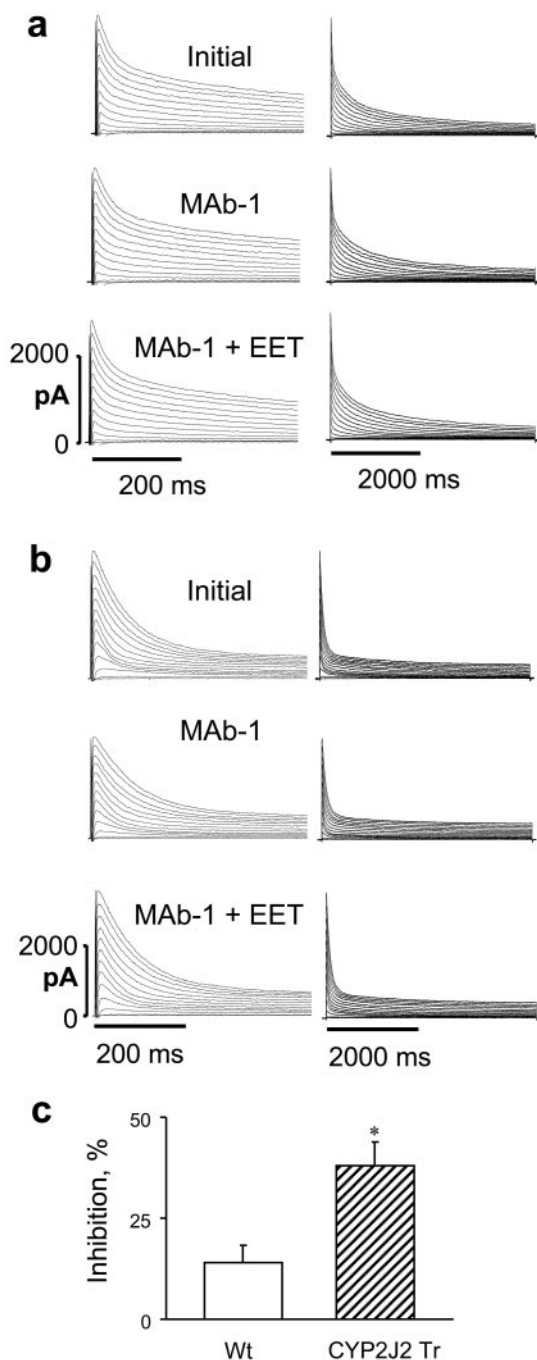| | $APD_{50}$ (ms) | | | | | | $APD_{90}$ (ms) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wt | | | CYP2J2 Tr | | | Wt | | | CYP2J2 Tr | | |
| | $n$ | Mean | SE | $n$ | Mean | SE | $n$ | Mean | SE | $n$ | Mean | SE |
| Control | 9 | 8.5 | 0.6 | 10 | 5.7 | 0.5 | 9 | 31.8 | 2.8 | 10 | 20.5 | 2.1 |
| MS-PPOH | 9 | 9.1 | 0.6 | 10 | 7.9* | 0.6 | 9 | 31.1 | 3.2 | 10 | 30.1* | 3.0 |
| cAMP | 9 | 9.1 | 0.7 | 10 | 6.2 | 0.7 | 9 | 33.4 | 3.9 | 10 | 23.2 | 2.2 |
| Initial | 9 | 9.2 | 0.6 | 9 | 5.2 | 0.6 | 9 | 33.2 | 3.6 | 9 | 21.5 | 2.5 |
| MAb-1 | 9 | 9.9 | 0.8 | 9 | 7.8* | 0.7 | 9 | 33.8 | 3.0 | 9 | 30.4** | 2.8 |
| cAMP | 9 | 9.0 | 1.0 | 9 | 6.5 | 0.8 | 9 | 33.1 | 3.3 | 9 | 25.6 | 2.3 |
| Initial | 9 | 8.8 | 0.7 | 9 | 5.9 | 0.7 | 9 | 30.8 | 3.2 | 9 | 19.8 | 2.2 |
| MAb-1 | 9 | 9.1 | 0.4 | 9 | 8.0* | 0.6 | 9 | 31.6 | 2.8 | 9 | 28.0* | 2.8 |
| EET | 9 | 8.8 | 0.5 | 9 | 6.7 | 0.7 | 9 | 30.9 | 2.5 | 9 | 22.1 | 2.4 |

* $p < 0.05$; ** $p < 0.01$.

**Fig. 4.** Suppression of $I_{to,peak}$ by an inhibitory CYP2J2 monoclonal antibody in CYP2J2 Tr cardiomyocytes. Whole-cell outward $K^+$ currents were evoked by 5-s (right) depolarizing pulses from −50 to +70 mV in 10-mV increments. The membrane holding potential was −60 mV, and pulse rate was 0.1 Hz. The early portions (600 ms) of the currents are shown in the corresponding left panels (see the protocols in Fig. 1b). a, the effect of intracellular dialysis with MAb-1 (0.125 mg/ml IgG) on the outward $K^+$ currents in a Wt cardiomyocyte. Addition of 40 nM 11,12-EET to the bath solution after MAb-1 dialysis did not significantly alter the outward $K^+$ currents. b, intracellular dialysis with MAb-1 (0.125 mg/ml IgG) significantly inhibited $I_{to,peak}$ but did not affect the late portion of $I_{to}$ ($I_{to,280ms}$) or the delayed outward $K^+$ current ($I_{k,slow}$) in a CYP2J2 Tr cardiomyocyte. Addition of 40 nM 11,12-EET to the bath solution reversed the MAb-1-induced inhibition of $I_{to,peak}$. c, averaged data from multiple experiments showing that inhibition of $I_{to,peak}$ by MAb-1 is significantly greater in CYP2J2 Tr cardiomyocytes than in Wt cardiomyocytes. Currents were evoked by 5-s single-step pulses from −60 to +70 mV. The pulse rate was 0.1 Hz with a holding potential of −60 mV. The maximal peak amplitude of $I_{to}$ was measured. Data are presented as mean ± S.E. ∗, $p < 0.05$ versus Wt.

tabolites of AA rather than by a direct interaction between the CYP2J2 protein and the $K^+$ channel, because MAb-1 is highly selective for inhibition of CYP2J2 activity but does not influence CYP2J2 protein levels. Therefore, enhancement of $I_{to,peak}$ and shortening of APD in CYP2J2 Tr mice most likely results from increased EET biosynthesis. It is noteworthy that we have previously reported that CYP2J2 Tr cardiomyocytes release significantly more stable EET products (DHETs) into culture media than do Wt cardiomyocytes (Seubert et al., 2004). These data are consistent with increased EET biosynthesis and the presence of an active epoxide hydrolase in CYP2J2 Tr cardiomyocytes.

CYP2J2-derived EETs may act through an intracellular signaling pathway that leads to channel phosphorylation (Xiao et al., 1998; Xiao, 2007). In this regard, we found that the effects of MS-PPOH and MAb-1 on $I_{to,peak}$ and APD were reversed by addition of the membrane permeable 8-Br-cAMP. These results are consistent with our previous findings that 11,12-EET increased intracellular cAMP levels and enhanced L-type $Ca^{2+}$ channel phosphorylation in rat cardiomyocytes (Xiao et al., 1998) and that overexpression of CYP2J2 in mouse cardiomyocytes significantly increased $I_{Ca}$
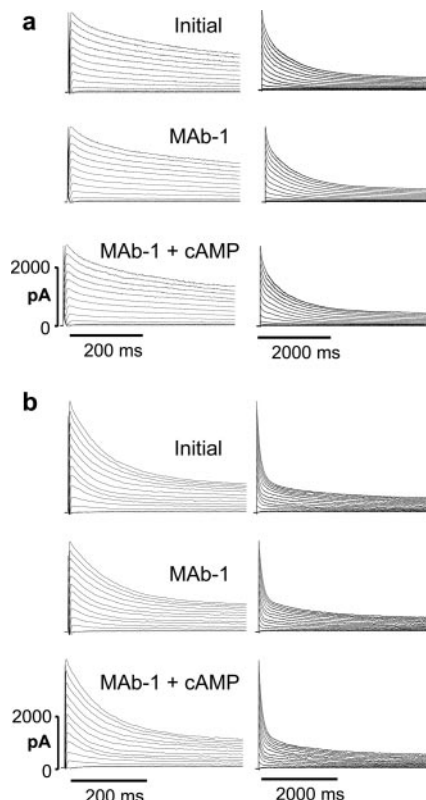


**Fig. 5.** Suppression of $I_{to,peak}$ by an inhibitory CYP2J2 monoclonal antibody in CYP2J2 Tr cardiomyocytes. Whole-cell outward $K^+$ currents were evoked by 5 s (right) depolarizing pulses from −50 to 70 mV in 10-mV increments. The membrane holding potential was −60 mV and pulse rate was 0.1 Hz. The early portions (600 ms) of the currents are shown in the corresponding left panels (see the protocols in Fig. 1b). a, the effect of intracellular dialysis with MAb-1 (0.125 mg/ml IgG) on the outward $K^+$ currents in a Wt cardiomyocyte. Addition of 2 mM 8-Br-cAMP to the bath solution after MAb-1 dialysis did not significantly alter the outward $K^+$ currents. b, intracellular dialysis with the MAb-1 (0.125 mg/ml IgG) significantly inhibited the peak $I_{to}$ ($I_{to,peak}$) but did not affect the late portion of $I_{to}$ ($I_{to,280ms}$) or the delayed outward $K^+$ current ($I_{k,slow}$) in a CYP2J2 Tr cardiomyocyte. Addition of 2 mM 8-Br-cAMP to the bath solution reversed the MAb-1-induced inhibition of $I_{to,peak}$.

via a cAMP-dependent mechanism (Xiao et al., 2004). Furthermore, the level of phosphorylated $\alpha_1$ subunit of the L-type $Ca^{2+}$ channel protein was significantly increased and inhibition of PKA activity significantly decreased $I_{Ca}$ in CYP2J2 Tr heart cells (Xiao et al., 2004). Together, these data suggest that CYP2J2-derived EETs act through a cAMP-PKA–dependent mechanism to enhance phosphorylation of the $\alpha_1$ subunit of the L-type $Ca^{2+}$ channel and increase $I_{Ca}$. Given that voltage-dependent $K^+$ channels such as Kv4.2 are also activated by cAMP-PKA-dependent phosphorylation of $\alpha$-subunits (Anderson et al., 2000), we speculate CYP2J2-derived EETs increase $I_{to,peak}$ via a similar mechanism.

If many ion channels are modulated by EETs, why was $I_{to,peak}$ preferentially affected in CYP2J2 Tr cardiomyocytes and not other channels, such as $I_{Na}$? While the precise mechanisms for this observation are unclear, one possibility is that the sensitivities of different ion channels to EETs may be different. For example, cardiac L-type $Ca^{2+}$ channels are also very sensitive to EETs (Xiao et al., 2004). Another possibility is that the sensitivity to and dependence on channel phosphorylation by the cAMP-PKA system may also vary among different types of ion channels.

Our previous data have shown that cardiac CYP2J2 overexpression enhances EET biosynthesis and improves postischemic recovery of left ventricular function (Seubert et al., 2004). Shortening of the cardiac action potential in CYP2J2 Tr cardiomyocytes may be one of the potential mechanisms for the beneficial effects of CYP2J2 overexpression on postischemic heart function. Indeed, some interventions that are cardioprotective (e.g., acute preconditioning, verapamil,
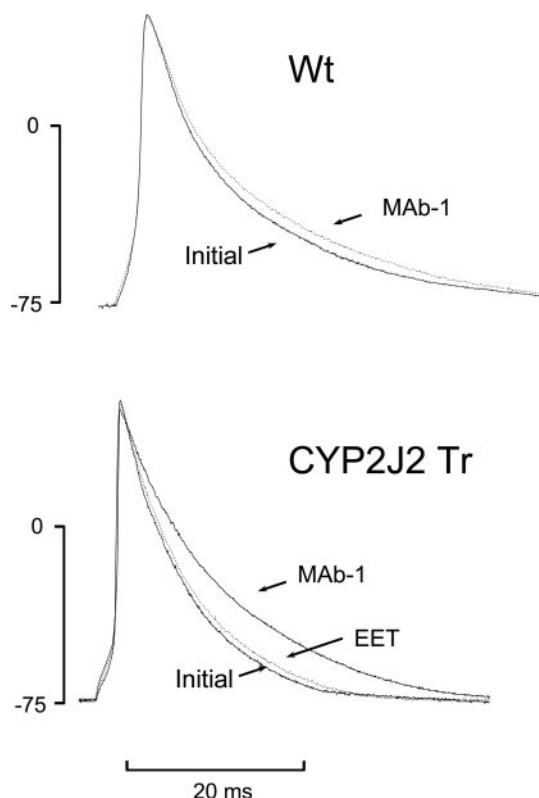
Fig. 6. Effects of an inhibitory CYP2J2 monoclonal antibody on action potentials in Wt and CYP2J2 Tr cardiomyocytes. Top, representative action potentials recorded from a ventricular myocyte isolated from a Wt mouse heart. Intracellular dialysis with the MAb-1 (0.125 mg/ml IgG) slightly prolonged the duration of the action potential. Bottom, intracellular dialysis with MAb-1 (0.125 mg/ml IgG) markedly prolonged the duration of the action potential in a CYP2J2 Tr cardiomyocyte. Bath perfusion with 40 nM 11,12-EET almost completely reversed the MAb-1 effect. The action potentials were elicited by intracellular injection of depolarizing current pulses (15 pA with 10 ms duration) from a holding membrane potential of approximately −75 mV. Initial, the action potentials were recorded immediately after forming the whole-cell configuration. MAb-1, the action potentials were recorded 15 min after intracellular dialysis with MAb-1. EET, the action potential were recorded 10 min after bath perfusion with 11,12-EET.
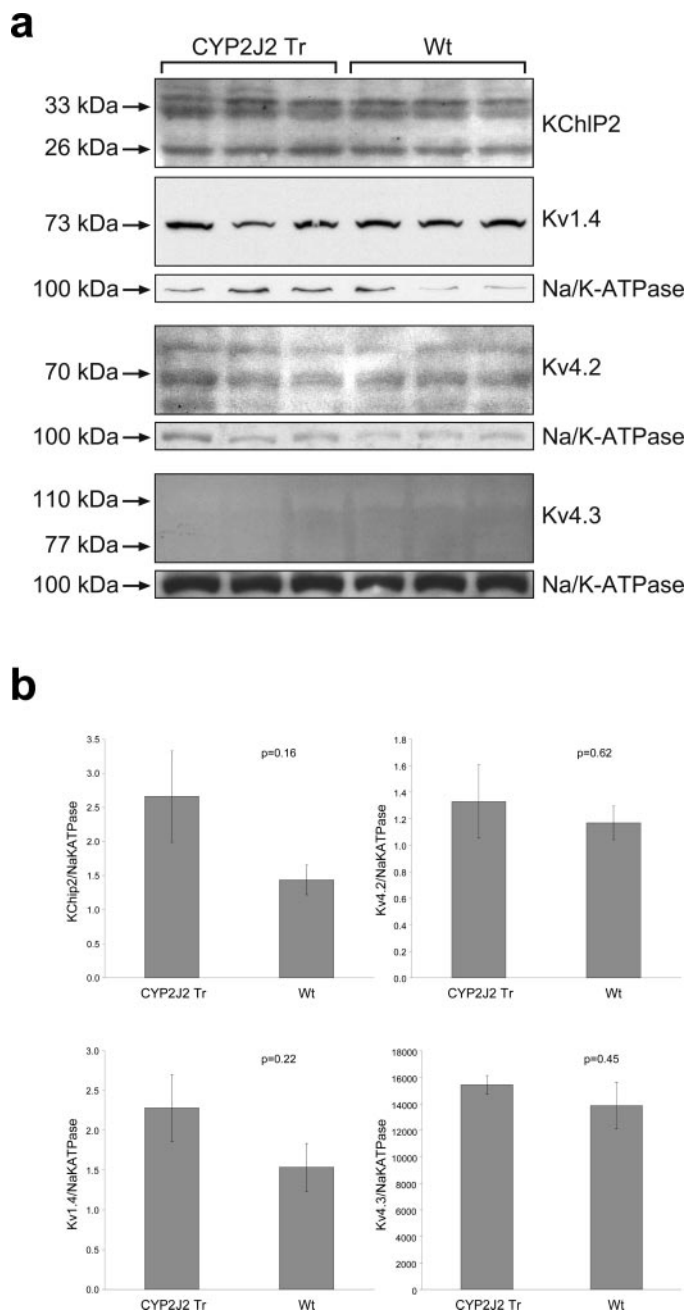
Fig. 7. Expression of voltage-gated $K^+$ channels in CYP2J2 Tr and Wt hearts. a, lysates prepared from CYP2J2 Tr ($n = 3$) and Wt ($n = 3$) hearts were immunoblotted with selective antibodies to KChIP2, Kv1.4, Kv4.2, Kv4.3, and $Na^+/K^+$-ATPase $\alpha1$ as described under *Materials and Methods*. Molecular masses are shown to the left of the panels. b, densitometry was performed and the expression of voltage-gated $K^+$ channels was normalized to the expression of $Na^+/K^+$-ATPase $\alpha1$.

$K_{ATP}$ channel openers) also shorten the cardiac action potential (Yao et al., 1993; Perchenet and Kreher, 1995). Decreasing action potential duration might limit $Ca^{2+}$ accumulation during ischemia resulting in reduced hypercontracture during reperfusion and result in improved postischemic recovery of left ventricular function (Steenbergen et al., 1993).

The effects of CYP2J2 overexpression on APD seem to be due primarily to increased maximal peak transient outward $K^+$ currents ($I_{to,peak}$), because the late portion of the transient outward $K^+$ current and the slowly inactivating $K^+$ current were similar in CYP2J2 Tr and Wt heart cells. Our previous finding that CYP2J2 Tr cardiomyocytes have enhanced $I_{Ca}$ (Xiao et al., 2004) seems to be inconsistent with our current findings of a shortened action potential in these cells, because an increase in $I_{Ca}$ would be expected to prolong APD. One possible explanation for this apparent contradiction is that $I_{to}$ may play a more dominant role in determining the duration of action potentials in mouse cardiomyocytes. The $I_{to,peak}$ density was $38.6 \pm 2.8$ pA/pF (Table 2), which is much greater than that of $I_{Ca}$ ($9.7 \pm 0.6$ pA/pF) in Wt cardiomyocytes (Xiao et al., 2004). In CYP2J2 Tr cardiomyocytes, the increase in $I_{to,peak}$ ($54.4 \pm 4.9$ pA/pF) is also much greater than the increase in $I_{Ca}$ ($13.6 \pm 0.9$ pA/pF). In addition, the fast inactivation time ($\tau$) is much slower for $I_{to}$ (74.4 ms for Wt and 47.4 ms for CYP2J2 Tr cardiomyocytes) (Table 3) than for $I_{Ca}$ (10.4 ms for the Wt and 10.0 ms for CYP2J2 Tr cardiomyocytes) (Xiao et al., 2004). Therefore, the larger increase in $I_{to,peak}$, but not the smaller enhancement in $I_{Ca}$, is the main cause of the shortened action potential in the CYP2J2 Tr cardiomyocytes.

Shortened APD may be arrhythmogenic and/or lead to sudden death. We evaluated electrocardiograms in conscious mice but found no significant differences in resting heart rate and no significant differences in spontaneous arrhythmias between CYP2J2 Tr and Wt mice (data not shown). Moreover, we have not observed significant differences in the incidence of sudden death between CYP2J2 Tr and Wt mice.

In summary, the major finding of this study is that the cardiac action potential was significantly shortened in CYP2J2 Tr cardiomyocytes and this shortening was probably due to an increase in $I_{to,peak}$. Moreover, our data suggest that CYP2J2-derived EETs affect APD and $I_{to,peak}$ via a cAMP-dependent mechanism. The EETs probably either directly or indirectly stimulate adenylyl cyclase and/or inhibit phosphodiesterase, leading to increased intracellular cAMP and enhanced cAMP-PKA dependent phosphorylation of the $K^+$ channel subunit. In this regard, two recent studies in noncardiac cells show that EETs enhance $Ca^{2+}$-activated $K^+$ currents via stimulation of $G_{s\alpha}$ in coronary vascular smooth muscle cells (Li and Campbell, 1997) and induce adenosine 2A receptor-mediated vasodilation of preglomerular microvessels via activation of a cAMP/PKA pathway (Carroll et al., 2006). In conclusion, CYP2J2-derived EETs may play an important role in the regulation of cardiac ion channels. In addition, shortening of the cardiac action potential in CYP2J2 Tr mice may contribute to improved recovery of heart contractile function after global ischemia.

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**Address correspondence to:** Dr. Darryl C. Zeldin, National Institutes of Health/NIEHS, 111 T. W. Alexander Drive, Building 101, Room D236, Research Triangle Park, NC 27709. E-mail: zeldin@niehs.nih.gov