Electrophysiological Properties of Cardiomyocytes Isolated from CYP2J2 Transgenic Mice

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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 ± 3.0 ms (n = 22) in wild-type (Wt) cells and 20.2 ± 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 (Ito,peak), which were 38.6 ± 2.8 and 54.4 ± 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 (Ito,280ms), the slowly inactivating outward K+ current (IK,slow), and the voltage-gated Na+ current (INa) were not significantly altered in CYP2J2 Tr cells. N-Methylsulphonyl-6-(2-proparglyoxy-phenyl)hexanamide (MS-PPOH), a specific inhibitor of EET biosynthesis, significantly reduced Ito,peak and increased APD in CYP2J2 Tr cardiomyocytes but not in Wt cells. Intracellular dialysis with a monoclonal antibody against CYP2J2 also significantly reduced Ito,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 Ito,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 Ito,peak via an EET-dependent, cAMP-mediated mechanism.

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ABBREVIATIONS: AA, arachidonic acid; EET, cis-epoxyeicosatrienoic acid; DHET, vic-dihydroxyeicosatrienoic acid; αMHC, α-myosin heavy chain; Tr, transgenic; Wt, wild type; MS-PPOH, N-methylsulfonyl-6-(2-proparglyoxyphenyl)hexanamide; MAb, monoclonal antibody against 8-Br-cAMP, 8-bromo-cAMP; APD, action potential duration; PKA, protein kinase A; Ito,peak, maximal peak transient outward K+ current; Ito,280ms, late portion of the transient outward K+ current; INa, voltage-gated Na+ current; KATP, 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 Ca²⁺ 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 Ca²⁺-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 Ca²⁺ channels in rat thymocytes (Alvarez et al., 1992) and in human neutrophils (Sargeant et al., 1992). EETs enhance L-type Ca²⁺ currents (I_{Ca,L}) 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 α-mysin heavy chain (α-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 Ca²⁺ 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-6,12-proparglyloxyphenylhexanamide (MS-PPOH), a specific inhibitor of EET biosynthesis, was synthesized as described previously (Wang et al., 1998). Working stocks of MS-PPOH (50 μM) were prepared in 100% ethanol and stored under argon at −20°C. 11,12-EET was prepared, a generous gift from Dr. Jeffrey Robbins (University of Cincinnati). This vector contains the αMHC 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 C57BL/6j 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 ~3 ml/min with oxygenated 37°C Tyrode’s solution containing 137 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 10 mM glucose, and 10 mM HEPES, pH 7.4. Hearts were then perfused with Ca²⁺-free Tyrode’s solution for 5 to 6 min, recirculated with Ca²⁺-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 μM CaCl₂ 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 μM CaCl₂, 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 10–20 MΩ 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 gigahm 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 ± 3.5 pF for WT cardiomyocytes \((n = 81)\) and 119.3 ± 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 −98% 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 CaCl₂. The pipette solution consisted of 90 mM potassium aspartate, 40 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 0.1 mM CaCl₂, 0.02 mM tetrodotoxin, 10 mM glucose, and 10 mM HEPES, pH 7.4. 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 MgCl₂, 2 mM CaCl₂, 0.1 mM CaCl₂, 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 MgCl₂, 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 MgCl₂, 2 mM CaCl₂, 0.1 mM CaCl₂, 10 mM glucose, and 10 mM HEPES, pH 7.4, and the pipette solution contained 100 mM CsCl, 40 mM CsOH, 1 mM MgCl₂, 1 mM CaCl₂, 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 8-week-old WT mice 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 α1 \((1:200 \text{ dilution})\) was performed according to the manufacturers’ instructions.

Statistical Analysis. The density (piconewton 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 trace (Xiao et al., 1998, 2004). The results of the steady-state inactivation of I_{Na} were fitted by a Boltzmann equation \((γ = 1/(1 + \exp(V - V_{0.5}/K}))\). The best-fit procedure was performed with a commercial software program (Origin 7.0; OriginLab Corp., Northampton, MA). All data are presented as mean ± 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⁺, Ca²⁺, 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 ± 1.5 mV in WT cardiomyocytes \((n = 41)\) and −62.6 ± 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; Nerboune, 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 \(\text{I}_{\text{to,peak}}\) 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 \(\text{I}_{\text{to,280ms}}\) or in the slowly inactivating K⁺ current \(\text{I}_{\text{K,slow}}\) (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 \(\tau_{\text{fast}}\) was 75.4 ± 3.9 ms for WT \((n = 18)\) and 47.5 ± 2.1 ms for CYP2J2 Tr \((n = 24)\) cells \((p < 0.001)\). The slow time constant \(\tau_{\text{slow}}\) was 1195 ± 58 ms for WT and 1233 ± 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 \(\text{I}_{\text{K1}}\) and \(\text{I}_{\text{K2}}\,\text{but~found~no~significant~differences~between~WT~and~CYP2J2~Tr~cardiomyocytes.}~\text{Maximal~inward~K⁺~currents~elicited~by~hyperpolarizing~pulses~(I}_{\text{K1}}\text{were~similar~in~CYP2J2~Tr~and~WT~cardiomyocytes~(Table~2).}~\text{Because~the~voltage-gated Na⁺~current~(I}_{\text{Na}}\text{could~also~affect~cardiac~APD,~we~compared~the~properties~of~I}_{\text{Na}}\text{in cardiomyocytes~isolated~from~WT~and~CYP2J2~Tr~mice~to~determine~whether~cardiac~overexpression~of~CYP2J2~altered~the~function~of~cardiomyocytes.}~\text{In~addition,~the~normalized~steady-state~inactivation~of~cardiomyocytes~isolated~from~WT~and~CYP2J2~Tr~mice~showed~that~cardiac~overexpression~of~CYP2J2~did~not~significantly~alter~the~function~of~the~voltage-gated~Na⁺~channels.}~\text{Effects~of~an~EET~ Biosynthesis~Inhibitor~on~I}_{\text{Na,peak}}\text{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 μM 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 (Ito,peak) was measured and averaged from individual cardiomyocytes (Wt, n = 22; CYP2J2 Tr, n = 23). Right, the rate of decay of transient outward K⁺ currents was measured at the point of 280 ms (Ito,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 < 0.01 versus Wt. d, slowly inactivating outward K⁺ currents (IK,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 (Ito), the fast inactivating component of the depolarization-activated K⁺ currents. Bottom, the current-voltage relationships of IK,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

<table>
<thead>
<tr>
<th>Mouse</th>
<th>Cell</th>
<th>APA (mV)</th>
<th>APT (pA)</th>
<th>Vmax (V/s)</th>
<th>APD50 (ms)</th>
<th>APD90 (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt</td>
<td>11</td>
<td>116.9 ± 5.1</td>
<td>22.9 ± 1.9</td>
<td>44.2 ± 4.9</td>
<td>8.65 ± 0.86</td>
<td>30.6 ± 3.0</td>
</tr>
<tr>
<td>CYP2J2 Tr</td>
<td>9</td>
<td>122.1 ± 6.2</td>
<td>21.1 ± 2.2</td>
<td>49.6 ± 6.3</td>
<td>5.86 ± 0.57*</td>
<td>20.2 ± 2.4*</td>
</tr>
</tbody>
</table>

APA, action potential amplitude; APT, action potential threshold (minimal depolarizing currents required for initiation of the first action potential); Vmax, maximum upstroke velocity; APD, action potential duration measured at 50% (APD50) and 90% (APD90) repolarization.

* p < 0.005 versus Wt.
rents ($I_{\text{to,peak}}$) 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 $I_{\text{to,peak}}$ 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 ($\tau_{\text{fast}}$) of outward $K^+$ currents in CYP2J2 Tr cardiomyocytes from 47.7 ± 2.0 to 68.5 ± 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 $I_{\text{to,peak}}$ 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 ± 2.1 ms (initial value) to 30.1 ± 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 $I_{\text{to,peak}}$ 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 non-CYP2J subfamily P450s, including members of the CYP1A, CYP1B, CYP2A, CYP2C, CYP2D, CYP2E, CYP3A, and CYP4A subfamilies (Xiao et al., 2004). To assess whether the enhanced cardiac $I_{\text{to,peak}}$ in CYP2J2 Tr mice were related to overexpression of CYP2J2, we internally di-

**TABLE 2**

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

<table>
<thead>
<tr>
<th></th>
<th>$I_{\text{to,peak}}$</th>
<th>$I_{\text{to,280 ms}}$</th>
<th>$I_{\text{K, slow}}$</th>
<th>$I_{\text{K1}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt</td>
<td>38.6 ± 2.8</td>
<td>24.3 ± 1.8</td>
<td>23.8 ± 1.6</td>
<td>-19.2 ± 2.4</td>
</tr>
<tr>
<td>(n = 22)</td>
<td>(n = 22)</td>
<td>(n = 22)</td>
<td>(n = 18)</td>
<td></td>
</tr>
<tr>
<td>CYP2J2 Tr</td>
<td>54.4 ± 4.9</td>
<td>26.3 ± 2.9</td>
<td>25.0 ± 2.5</td>
<td>-18.3 ± 2.0</td>
</tr>
<tr>
<td>(n = 23)</td>
<td>(n = 23)</td>
<td>(n = 24)</td>
<td>(n = 12)</td>
<td></td>
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</table>

* $p < 0.01$ versus Wt.

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 (○, $n = 15$) and CYP2J2 Tr (●, $n = 11$) 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 (○) and CYP2J2 Tr (●) cardiomyocytes. The inactivation data points of peak $I_{\text{K1}}$ were fitted to a Boltzmann equation.
whole-cell configuration was taken as the control value. $I_{\text{to-peak}}$ gradually decreased after intracellular dialysis with MAb-1 in CYP2J2 Tr cardiomyocytes (Figs. 4 and 5); however, the inhibition of $I_{\text{to-peak}}$ reached statistical significance only in CYP2J2 Tr cardiomyocytes (Figs. 4c, Table 3). Thus, $I_{\text{to-peak}}$ 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 $I_{\text{to-peak}}$ 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 $I_{\text{to-peak}}$.

The inhibition of $I_{\text{to-peak}}$ 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 $I_{\text{to-peak}}$ in CYP2J2 Tr cardiomyocytes dialyzed with MAB-1 (Figs. 4 and 5). Together, these results indicate that selective inhibition of CYP2J2 activity with MAB-1 results in a significant reduction of cardiomyocyte $I_{\text{to-peak}}$ 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 ± 2.5 ms (initial value) to 30.4 ± 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 $I_{\text{to-peak}}$ 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 either Wt or CYP2J2 Tr mice. 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.

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**Fig. 3.** Suppression of $I_{\text{to-peak}}$ 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 μM 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 μM MS-PPOH significantly inhibited $I_{\text{to-peak}}$, but did not affect the late portion of $I_{\text{to}}$ ($I_{\text{to,280ms}}$) or the delayed outward K⁺ current ($I_{\text{to,280ms}}$) in a CYP2J2 Tr cardiomyocyte. Addition of 2 mM 8-Br-cAMP reversed the MS-PPOH-induced inhibition of $I_{\text{to-peak}}$. c, averaged data from multiple experiments showing inhibition of $I_{\text{to-peak}}$ by 25 μM 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 $I_{\text{to}}$ was measured. Data are presented as mean ± S.E. **p < 0.01 versus Wt.
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, 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 cardioprotective (Nithipatikom et al., 2001, 2006; 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-
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 Ito,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 Ito,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 Ca2+ channel phosphorylation in rat cardiomyocytes (Xiao et al., 1998) and that overexpression of CYP2J2 in mouse cardiomyocytes significantly increased ICSa.

Fig. 4. Suppression of Ito,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 Ito,peak but did not affect the late portion of Ito (Ito,280ms) or the delayed outward K+ current (Ik,slow) in a CYP2J2 Tr cardiomyocyte. Addition of 40 nM 11,12-EET to the bath solution reversed the MAb-1-induced inhibition of Ito,peak. c, averaged data from multiple experiments showing that inhibition of Ito,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 Ito was measured. Data are presented as mean ± S.E. *, p < 0.05 versus Wt.

Fig. 5. Suppression of Ito,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 MAb-1 (0.125 mg/ml IgG) significantly inhibited the peak Ito (Ito,peak) but did not affect the late portion of Ito (Ito,280ms) or the delayed outward K+ current (Ik,slow) in a CYP2J2 Tr cardiomyocyte. Addition of 2 mM 8-Br-cAMP to the bath solution reversed the MAb-1-induced inhibition of Ito,peak.
via a cAMP-dependent mechanism (Xiao et al., 2004). Furthermore, the level of phosphorylated α-subunit of the L-type Ca2+ channel protein was significantly increased and inhibition of PKA activity significantly decreased ICa 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 α-subunit of the L-type Ca2+ channel and increase ICa. Given that voltage-dependent K+ channels such as Kv4.2 are also activated by cAMP-PKA-dependent phosphorylation of α-subunits (Anderson et al., 2000), we speculate CYP2J2-derived EETs increase ICa via a similar mechanism.

If many ion channels are modulated by EETs, why was Ito,peak preferentially affected in CYP2J2 Tr cardiomyocytes and not other channels, such as INa? 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 Ca2+ 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, atropine).

**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 α1 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 α1.
K_{\text{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 (Steinberg et al., 1993).

The effects of CYP2J2 overexpression on APD seem to be due primarily to increased maximal peak transient outward K+ currents (I_{\text{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_{\text{Ca_L}} (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_{\text{Ca_L}} would be expected to prolong APD. One possible explanation for this apparent contradiction is that I_{\text{Ca_L}} may play a more dominant role in determining the duration of action potentials in mouse cardiomyocytes. The I_{\text{to_peak}} density was 38.6 ± 2.8 pA/pF (Table 2), which is much greater than that of I_{\text{Ca_L}} (9.7 ± 0.6 pA/pF) in WT cardiomyocytes (Xiao et al., 2004). In CYP2J2 Tr cardiomyocytes, the increase in I_{\text{to_peak}} (54.4 ± 4.9 pA/pF) is also much greater than the increase in I_{\text{Ca_L}} (13.6 ± 0.9 pA/pF). In addition, the fast inactivation time (\tau_i) is much slower for I_{\text{to}} (74.4 ms for WT and 47.4 ms for CYP2J2 Tr cardiomyocytes) (Table 3) than for I_{\text{Ca_L}} (10.4 ms for the WT and 10.0 ms for CYP2J2 Tr cardiomyocytes) (Xiao et al., 2004). Therefore, the larger increase in I_{\text{to_peak}}, but not the smaller enhancement in I_{\text{Ca_L}}, 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_{\text{to_peak}}. Moreover, our data suggest that CYP2J2-derived EETs affect APD and I_{\text{to_peak}} via a cAMP-dependent mechanism. The EETs probably either directly or indirectly stimulate adenyl 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 non-cardiomyocytes show that EETs enhance Ca^{2+}-activated K+ currents via stimulation of G_{\alphaq} 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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