Stereospecific Activation of Cardiac ATP-Sensitive K⁺ Channels by Epoxyeicosatrienoic Acids: A Structural Determinant Study

TONG LU, MIKE VANROLLINS, and HON-CHI LEE

Departments of Internal Medicine, Mayo Clinic, Rochester, Minnesota (T.L., H.L.), and the University of Iowa, Iowa City, Iowa (M.V.)

Received February 21, 2002; accepted July 16, 2002 This article is available online at http://molpharm.aspetjournals.org

ABSTRACT

The heart is richly endowed with K_\(\text{ATP}\) channels, which function as biological sensors, regulating membrane potentials and electrical excitability in response to metabolic alterations. We recently reported that the cytochrome P450 metabolites of arachidonic acid, epoxyeicosatrienoic acids (EETs), potently activate cardiac K_\(\text{ATP}\) channels by reducing channel sensitivity to ATP. In the present study, we further demonstrate that 11(S),12(R)-EET activated the cardiac K_\(\text{ATP}\) channels with an EC_{50} of 39.5 nM, whereas 11(R),12(S)-EET was totally inactive. In addition, 11(S),12(R)-EET but not 11(R),12(S)-EET hyperpolarized the resting membrane potentials and shortened the duration of cardiomyocyte action potentials. By studying homologs and analogs of 11,12-EET, we also found that all four EET regioisomers are equipotent activators of the K_\(\text{ATP}\) channels, reducing the ATP sensitivity by more than 10-fold; however, neither altered chain length, double bond number, epoxide position, nor methylation of the carboxyl group affected channel inhibitions by ATP. All the fatty epoxides studied are potent K_\(\text{ATP}\) channel activators, but the \(\omega\)-3 homolog was particularly potent, reducing ATP sensitivity 27-fold. Together, the results indicate that the presence of an epoxide group in a particular three-dimensional configuration is a critical determinant for K_\(\text{ATP}\) channel activation, and its effect is augmented by a double bond at \(\omega\)-3 position. The results also suggest that fatty epoxides are important modulators of cardiac electrical excitability.

ATP-sensitive K⁺ (K_\(\text{ATP}\)) channels are densely populated in the heart and play an important role in regulating the membrane potential in response to intracellular metabolic oscillations (Yokoshiki et al., 1998). K_\(\text{ATP}\) channels are thought to be activated during cardiac ischemia and hypoxia when cytoplasmic ATP is depleted, which would lead to shortened cardiac action potentials, reducing Ca²⁺ influx and minimizing intracellular Ca²⁺ overload (Noma, 1983; Nichols and Lederer, 1990; Tung and Kurachi, 1991). However, the function of the cardiac K_\(\text{ATP}\) channels under normal conditions is not well understood.

Arachidonic acid is an important precursor of many bioactive lipids (Brash, 2001; Funk, 2001; Roman, 2002). The cytochrome P450 (P450) epoxide enzyme metabolites of arachidonic acid have emerged as an important source of signaling agents (Zeldin, 2001; Roman, 2002). The CYP2J epoxides, abundant in human and rat hearts (Zeldin, 2001), convert arachidonic acid into four epoxyeicosatrienoic acid (EET) regioisomers: 5,6-, 8,9-, 11,12-, and 14,15-EET, each of which can be formed as the \(RS\) or \(SR\) enantiomer (McGiff, 1991; Zeldin, 2001). EET products are endogenous constituents of rat cardiac tissue, occurring at 69 ± 7 ng of total EETs/g of heart (Wu et al., 1997). EETs can be further hydrolyzed to dihydroxyeicosatrienoic acids (DHETs) by epoxide hydrolase (Zeldin et al., 1993). In coronary vascular endothelial cells, inhibition of soluble epoxide hydrolase results in the accumulation of both chain elongation and chain shortened homologs of EET (Fang et al., 2001).

There is very limited information on the specific binding of EETs in cells and an EET receptor has not yet been identified. Most of the physiological effects of EETs do not appear to involve regio- or stereospecificity (Oltman et al., 1998; Lee et al., 1999; Lu et al., 2001b; Zhang et al., 2001). However, specific high affinity binding sites for 14,15-EET are present in U-937 cells, which may be associated with a receptor (Wong et al., 1997), and stereospecific effects of 11,12-EET have been reported on renal vascular tone and K⁺ channel activity (Zou et al., 1996). Nevertheless, stereospecific effects

ABBREVIATIONS: K_\(\text{ATP}\) channels, ATP-sensitive K⁺ channels; P450, cytochrome P450; EET, epoxyeicosatrienoic acid; DHET, dihydroxyeicosatrienoic acid; EDT, epoxycosatrienoic acid; EEQ, epoxycosaquatraenoic acid; APD_{90}, action potential duration at 90% repolarization; RP, resting membrane potential; BK channels, large conductance Ca²⁺-activated potassium channels.
of EETs have not been previously demonstrated in cardiac tissue.

Recently, we reported that EETs potently activated the cardiac K<sub>ATP</sub> channels by reducing channel sensitivity to ATP, allowing the channel to remain open in the presence of physiological concentrations of cytoplasmic ATP (5 mM) (Lu et al., 2001a). In the present study, we tried to determine whether EETs stereospecifically activated K<sub>ATP</sub> channels and stereospecifically altered the membrane potentials of cardiac myocytes. In addition, to assess the role of other structural determinants, we examined the effects of various EET homologs and analogs on the K<sub>ATP</sub> channel activity (Fig. 1). Although epoxide stereochemistry was critical for K<sub>ATP</sub> channel activation and membrane potential hyperpolarization, the position of the epoxide group and the presence of an anionic carboxylic group were not. These results suggest that endogenous EETs may activate cardiac K<sub>ATP</sub> channels by binding at specific sites in cardiomyocytes, thereby modulating cardiac electrophysiology.

**Materials and Methods**

**Preparation of Single Ventricular Myocytes.** Ventricular myocytes were isolated from male Sprague-Dawley rats by enzymatic dissociation (Lee et al., 1999). Briefly, animals were anesthetized with methoxyfluorane and the hearts were rapidly excised. A modified Langendorff apparatus was used to perfuse hearts for 5 min at 37°C with nominally Ca<sup>2+</sup>-free Tyrode’s solution containing 138 mM NaCl, 4.5 mM KCl, 0.5 mM MgCl<sub>2</sub>, 0.33 mM Na<sub>2</sub>HPO<sub>4</sub>, 5.5 mM glucose, 10 mM HEPES, and 0.1% (w/v) bovine serum albumin, adjusted to pH 7.4 with NaOH. Isolated ventricular myocytes were placed in a 0.5-ml chamber, and positioned on the stage of an inverted microscope (Olympus, IX70, Japan) before being superfused at 2 ml/min with a solution containing 70 mM KCl, 70 mM L-aspartic acid, 20 mM taurine, 0.5 mM MgCl<sub>2</sub>, 1.0 mM K<sub>2</sub>HPO<sub>4</sub>, pH to 7.38 with KOH. Dissociated cells were filtered through a medium mesh, centrifuged, and used within 24 h.

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where $S$ represents the concentration of the chemical, $EC_{50}$ is the concentration at half-maximal effect, and $H$ is the Hill coefficient. Curve fittings were performed using Igor 3.16 software (WaveMetrics Inc., Lake Oswego, OR).

**Whole Cell Current Clamp Recordings.** Action potentials in rat ventricular myocytes were recorded at 37°C using the whole cell current clamp technique. Action potentials were elicited using 1 nA stimuli at 0.5 Hz and pCLAMP software. The signals were recorded with an Axopatch 200B amplifier, filtered at 5 kHz, and digitized at 20 kHz. The effects of 11(R),12(S)-EET, 11(S),12(R)-EET, and 11(S),12(R)-EET plus glyburide on the resting membrane potential (RP), action potential amplitude, and action potential duration at 90% repolarization ($APD_{90}$) were measured. For these measurements, the cells were superfused with a Ca$^{2+}$-Tyrode’s solution containing 138 mM NaCl, 4.5 mM KCl, 0.5 mM MgCl$_2$, 1.8 mM CaCl$_2$, 0.33 mM Na$_2$HPO$_4$, 5.5 mM glucose, 10 mM HEPES, pH adjusted to 7.4 with NaOH. The pipette solution contained 130 mM KCl, 1.0 mM EGTA, 10 mM HEPES, 1.0 mM MgCl$_2$, 5 mM ATP, 1.0 mM GTP, 0.018 mM CaCl$_2$, pH to 7.38 with KOH.

**Materials.** All chemicals used were racemic mixtures unless mentioned otherwise. The 1-14C-labeled EET regioisomers, 13,14-EDT, 11,12-EET, and the 11,12-EET enantiomers were freshly synthesized as previously described (Zhang et al., 2001). Arachidonic acid was purchased from Nu-Chek-Prep (Elysian, MN), 11,12-EET methyl ester, 11,12-DHET, 12,13-leukotxin, and linoleic acid were obtained from Cayman Chemical (Ann Arbor, MI). Lactobacillic acid was purchased from Matreya Inc. (State College, PA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Arachidonic acid, EETs, 11,12-EET methyl ester, 12,13-leukotxin, 13,14-EDT, linoleic acid, and lactobacillic acid were prepared in 100% ethanol (5 mM) and stored under nitrogen at −20°C. Glyburide was dissolved in dimethylsulfoxide and stored at −20°C. The chemical stocks were used at greater than or equal to 1:103 dilutions in the experiments, and the final concentrations of ethanol or dimethylsulfoxide were less than 0.1%.

**Statistical Methods.** Data are presented as mean ± S.E.M. Student's $t$ test or paired $t$ test was used to compare data between two groups. One-way analysis of variance with Tukey’s test was used to compare data from multiple groups using Sigma Stat 2.0 software (Jandel Co., San Rafael, CA). Statistical significance was assumed at $p < 0.05$.

**Results**

Because 5 μM 11,12-EET activates the K$_{ATP}$ channels by reducing the channel sensitivity to ATP (Lu et al., 2001a), we compared the effects of 5 μM of various EET homologs and analogs. The results on EET regioselectivity are shown in Fig. 2. Figure 2A shows recordings of K$_{ATP}$ channels at −60 mV in the presence of 1 mM cytoplasmic ATP, with or without 5 μM 5,6-, 8,9-, 11,12-, and 14,15-EET. ATP (1 mM) was used because it almost totally (99%) suppressed K$_{ATP}$ channel activities. However, in the presence of 5 μM EETs, appreciable K$_{ATP}$ channel activity was evident. The K$_{ATP}$ channel $P_o$ versus ATP concentration relationships in the presence and absence of 5 μM EET regioisomers are plotted in Fig. 2B. All EET regioisomers were potent in reducing ATP sensitivity and increased the ATP IC$_{50}$ from 31.5 ± 7.3 (n = 7) to 306 ± 52 μM for 5,6-EET (n = 7, $p < 0.05$ versus control), to 315 ± 36 μM for 8,9-EET (n = 6, $p = 0.05$ versus control), to 366 ± 72 μM for 11,12-EET (n = 7, $p < 0.05$ versus control), to 498 ± 83 for 14,15-EET (n = 6, $p < 0.05$ versus control), and without significant changes in the Hill coefficient of 1 to 1.2. Thus, the position of the epoxide group was not critical for K$_{ATP}$ activation. The ATP IC$_{50}$ values for the EETs in this study were two-fold lower than previously reported (Lu et al., 2001a). We believe this difference could be accounted for by the source of EETs. In our previous study, we obtained 11,12-EET from a commercial source, whereas in the present study, [1-14C]EETs were synthesized and the final concentrations were carefully checked by liquid scintillation techniques.

EET homologs and analogs (Fig. 1) were also tested to delineate whether other structural groups were important for EET activation of the K$_{ATP}$ channels (Fig. 3). Arachidonate and 11,12-DHET, the respective precursor and product...
of 11,12-EET, had no effect on the KATP channel activity; the ATP IC50 remained at 43.7 ± 10.1 μM (n = 6, p = N.S. versus control) and 39.5 ± 11.3 μM (n = 7, p = N.S. versus control), respectively (Fig. 3A). These results suggest that EET activation of the KATP channels in cardiac myocytes are not due to nonspecific fatty acid effects. In contrast, the methyl ester of 11,12-EET produced an effect similar to that of unesterified 11,12-EET; the ATP IC50 was shifted to 453 ± 110 μM (n = 6, p = N.S. versus 11,12-EET), and the Hill coefficient (1.0) remained unaltered (Fig. 3B). Thus, the presence of the epoxide group but not that of the anionic carboxylic group is crucial for KATP channel activation.

EET homologs and analogs were also used to test the effects of chain length on KATP channel activation. 12,13-Leukotoxin (9E,12,13-cis-epoxy-18:1), an 18-carbon epoxide with two less double bonds than the EETs, activated the KATP channel and increased the ATP IC50 of the channel to 398 ± 79 μM (n = 6, p < 0.05 versus control), similar to those of EETs (Fig. 3C). In contrast, lactobacillic acid (cis-12,13-methylene 18:0), an analog of 12,13-leukotoxin, which contains a cis methylene ring instead of a cis oxirane ring and no double bond, had no effect on the ATP IC50 (34.3 ± 13.1 μM, n = 7, p = N.S. versus control). Furthermore, the unsubstituted fatty acid analog, linoleic acid (9E,12E-18:2), had no effect on the KATP channel activation with ATP IC50 at 34.1 ± 12.5 μM (n = 6, p = N.S. versus control). These results confirmed that the epoxide group is critical for KATP channel activation, whereas chain shortening by two carbons or reducing the number of double bonds did not alter the epoxide effect.

EET homologs and analogs were also used to test the effects of chain elongation or increasing the number of double bonds (Fig. 3D). 13,14-EDT is an 11,12-EET chain-elongation homolog with two additional carbons inserted at the COOH end. 11,12-EEQ is an ω-3 analog of 11,12-EET with an additional double bond between 17-C and 18-C (Fig. 1). 13,14-EDT was as effective as 11,12-EET in reducing the KATP channel sensitivity to ATP; the IC50 shifted to 321 ± 64 μM (n = 6, p < 0.05 versus control; p = N.S. versus 11,12-EET). Surprisingly, 11,12-EEQ was more potent than 11,12-EET in reducing ATP sensitivity in the KATP channel; yet, although 11,12-EEQ shifted the IC50 to 1071 ± 261 μM (n = 5, p < 0.05 versus 11,12-EET), the Hill coefficient remained unaltered at 1.1. These results confirmed that small (2 carbon) changes in chain-length were not critical; however, an additional double bond at the ω-3 position enhanced the ability of the epoxide to activate the KATP channels.

To determine whether 11,12-EET stereospecifically activated KATP channels, we synthesized and tested both of the 11(R),12(S)-EET and 11(S),12(R)-EET enantiomers. Figure 4A shows recordings of multiple KATP channels in the presence of 5 μM 11(S),12(R)-EET (left) and 11(R),12(S)-EET (right). In the presence of 1.0 mM cytoplasmic ATP, KATP channel activity was significantly enhanced by 11(S),12(R)-EET but not by 11(R),12(S)-EET. 11(S),12(R)-EET shifted the ATP inhibitory curve to the right. The IC50 of 329 ± 124 μM (n = 5, p < 0.05 versus control) and Hill coefficient of 1.2 matched that of racemic 11,12-EET. In contrast, 11(R),12(S)-EET had no effect; the IC50 remained at 32.3 ± 11.2 μM (n = 5, p = N.S. versus control; p < 0.05 versus 11(S),12(R)-EET) (Fig. 4B). Thus, the three-dimensional presentation of the epoxide was critical for activating KATP channels.

The potency of 11(S),12(R)-EET in activating KATP channels was estimated by measuring the EC50 for channel activation. Channel activity was determined at −60 mV and with 100 μM cytosolic ATP because submaximal (only 80%) KATP channel activity was inhibited under these conditions. 11(S),12(R)-EET dose dependently enhanced the KATP channel activities (Fig. 5A, left column), whereas 11(R),12(S)-EET was inactive (Fig. 5A, right column). 11(S),12(R)-EET...
potently activates the \(K_{ATP}\) channel with an \(EC_{50}\) of 39.5 ± 12.4 nM \((n = 5)\), and this was about 2-fold higher than that previously reported for 11,12-EET (Lu et al., 2001a). We believe the differences in the source of EETs may account for the difference in results. In contrast, 11(R),12(S)-EET did not change channel activity at any concentration tested (Fig. 6B).

To determine whether activation of the \(K_{ATP}\) channel altered the membrane potential in whole cells, we examined the effects of the 11,12-EET enantiomers on action potentials recorded from isolated ventricular myocytes. One typical experiment is shown in Fig. 6A. Group data are shown in Fig. 6, B and C. Superfusion of 5 \(\mu\)M 11(S),12(R)-EET hyperpolarized the RP from \(-79.3 ± 2.3\ mV\) to \(-83.4 ± 2.6\ mV\) \((n = 8, p = 0.008)\) and shortened the APD\(_{90}\) from 41.6 ± 5.0 ms to 35.5 ± 4.8 ms \((n = 8, p = 0.006)\). The amplitude of the action potential at baseline \((104.1 ± 5.5\ mV)\) was not changed by 11(S),12(R)-EET \((102.2 ± 5.7\ mV, n = 8, p = N.S.)\). Both the changes in RP and APD\(_{90}\) were blocked by 2 \(\mu\)M glyburide, suggesting that the 11(S),12(R)-EET effects were due to \(K_{ATP}\) channel activation (Fig. 6B). However, superfusion of 5 \(\mu\)M 11(R),12(S)-EET did not alter the RP \((-84.5 ± 1.4\ mV\) versus \(-83.5 ± 1.9\ mV\) at baseline, \(n = 8, p = N.S.)\), the APD\(_{90}\) \((42.7 ± 3.7\ ms\) versus \(42.2 ± 2.3\ ms\) at baseline, \(n = 8, p = N.S.)\), or the action potential amplitude \((101.6 ± 3.6\ mV\) versus \(101.0 ± 2.3\ mV\) at baseline, \(n = 8, p = N.S.)\) (Fig. 6C). These results indicate that modulation of the cardiac action potential by 11,12-EET is stereospecific.

### Discussion

In the present study, we have made four important and novel observations. First, we have demonstrated that activation of the \(K_{ATP}\) channel by EET involves absolute stereospecificity; only the 11(S),12(R)-EET enantiomer is active. Second, the epoxide group on EET is a critical structural determinant for activation of the \(K_{ATP}\) channels. Third, an \(\omega-3\) double bond enhances the potency of the epoxide for \(K_{ATP}\) channel activation. Fourth, 11(S),12(R)-EET hyperpolarizes resting membrane potentials and shortens action potentials. These results suggest that endogenous EETs are specific activators of the cardiac \(K_{ATP}\) channels and may play an important role in regulating cardiac electrical excitability.

EETs regulate vital physiological functions including vasoreactivity (Campbell et al., 1996; Oltman et al., 1998; Zhang et al., 2001), inflammatory responses (Node et al., 1999), and cell proliferation (Fleming et al., 2001). Ion channels constitute an important class of EET effectors. In blood vessels, EETs cause vasodilation by activating the large con-

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**Fig. 4.** Stereospecific effect of 11,12-EET on \(K_{ATP}\) channel activity. Experiments were performed and \(P_o\) normalized as described in Fig. 2B. A, current tracings of \(K_{ATP}\) channels in the presence of 5 \(\mu\)M 11(S),12(R)-EET (left) or 5 \(\mu\)M 11(R),12(S)-EET (right) and varying concentrations of ATP. B, relationships of normalized \(P_o\) plotted against cytoplasmic ATP concentrations in the absence (open circles) and presence (filled circles) of 5 \(\mu\)M 11(S),12(R)-EET or 5 \(\mu\)M 11(R),12(S)-EET (filled squares). \(n = 5\) for both groups.

**Fig. 5.** Dose-dependent effect of 11(S),12(R)-EET on \(K_{ATP}\) channel activation. A, raw tracings of \(K_{ATP}\) channel currents were recorded at \(-60\ mV\) with 0.1 \(\mu\)M ATP, 1 \(\mu\)M Ca\(^{2+}\), and in the presence of various concentrations of 11(S),12(R)-EET (left) and 11(R),12(S)-EET (right). B, 11(S),12(R)-EET activated cardiac \(K_{ATP}\) channel with an \(EC_{50}\) of 39.5 ± 12.4 nM (filled circles), whereas 11(R),12(S)-EET was totally inactive (filled squares). \(n = 5\) for both groups.
Fig. 6. Stereospecific effect of 11,12-EET on action potentials in isolated ventricular myocytes. A, action potentials were elicited from a single ventricular myocyte at 37°C using current clamp techniques with 5 mM ATP in the pipette. RP was hyperpolarized and APD₉₀ was shortened on exposure to 5 μM 11(S),12(R)-EET and these effects were reversed by superfusion with 2 μM glyburide. 11(R),12(S)-EET had no effect. Scatter plots of group data are shown for 11(S),12(R)-EET (n = 8) (B) and for 11(R),12(S)-EET (n = 8) (C). The dark solid symbols and lines represent the means ± S.E.M. *, p < 0.05 versus baseline.
ductance Ca$^{2+}$-activated K$^+$ (BK) channels and are putative endothelium-derived hyperpolarizing factors (Li and Campbell, 1997; Campbell and Harder, 1999). EETs are endogenous constituents of the heart (Wu et al., 1997). Moreover, EETs inhibit cardiac Na$^+$ channels with the characteristics of a “modulated receptor” mechanism (Lee et al., 1999). The effects of EETs on the cardiac L-type Ca$^{2+}$ channel are more variable, and both stimulation (Xiao et al., 1998) and inhibition (Chen et al., 1999) of channel activity have been observed. Recently, we reported that EETs are potent activators of the cardiac K$_{ATP}$ channels, capable of activating the channel in the presence of 5 mM ATP, with an EC$_{50}$ of $10^{-8}$ M (Lu et al., 2001a). The present study confirms that EETs are important K$_{ATP}$ channel activators. We believe that the stereospecific effects of 11,12-EET on the K$_{ATP}$ channel indicate specific interaction between 11,12-EET and the K$_{ATP}$ channel protein or a channel-associated protein. It is likely that such specificity would entail a physiologically relevant and important function. The structurally specific requirements of the EET molecule for activating myocardial K$_{ATP}$ channels is in sharp contrast to the relative lack of structural specificity for activating BK channels in coronary blood vessels (Zhang et al., 2001). EET-induced dilation and activation of BK channels in coronary microvessels did not show stereospecificity (Zhang et al., 2001). Also, 11,12-DHET is as potent as 11,12-EET in producing coronary vasorelaxation (Oltman et al., 1998) and in activating coronary BK channels (Lu et al., 2001b). To date, the stereospecificity of EET-induced vascular effects has been restricted to platelets (Fitzpatrick et al., 1986) and renal vessels (Katoh et al., 1991; Zou et al., 1996). Interestingly, 11(R),12(S)-EET rather than 11(S),12(R)-EET activates the BK channels in renal arteriolar smooth muscle cells (Zou et al., 1996), suggesting that stereospecificity is unique for different channel proteins.

Only limited data suggest EETs bind to specific receptors; however, binding of EETs to these sites has not yet been shown to alter cellular function (Wong et al., 1997). Fatty acid epoxides interact enantioselectively with many proteins including P450 epoxygenases and epoxide hydrolases (Orru and Faber, 1999). In the present study, the epoxide is a critical functional group in EETs, which activates K$_{ATP}$ channels. Interaction between the oxirane ring and specific residues on the K$_{ATP}$ channel conceivably requires precise configuration and orientation, thereby producing specific conformational changes in the channel protein leading to reduced ATP sensitivity and enhanced channel activity. Because substitution of the epoxide with diols or with a methylene ring abolished the K$_{ATP}$ effects, the oxirane ring oxygen may facilitate critical hydrogen bond bridges or form an “intermediate” with nucleophilic groups of the K$_{ATP}$ channel. However, formation of stable intermediates is unlikely because the effects of EETs on the K$_{ATP}$ channel were rapidly reversible upon drug washout. Perhaps more importantly, the present findings exclude the possibility that EETs activate the K$_{ATP}$ channels via a mechanism similar to that by phosphoinositides, in which the positively charged residues at the COOH terminus of the K$_{ATP}$ channel are tethered by the electrostatic force of the anionic groups of the phospholipids at the cytoplasmic surface of the membrane, allowing the channel to be active (Fan and Makieleski, 1997). Such a mechanism would require neither stereospecificity nor the presence of an epoxide group. Epoxide-based modulation of protein function has received increasing attention, and the utilization of epoxides in drug design has been shown to be effective in a number of compounds including a novel antitumor antibiotic, Rhizoxin (Tolcher et al., 2000) and an angiogenesis inhibitor, TNF-470 (Stadler et al., 1999).

$\omega$-3 fatty acids are essential fatty acids enriched in fish oils and have been shown to be cardioprotective from their anti-thrombosis, anti-atherosclerosis, anti-inflammatory, anti-vasoconstriction, and anti-arrhythmic effects (Connor, 2000). Kang and Leaf (2000) suggest that the suppression of fatal cardiac arrhythmias by $\omega$-3 polyunsaturated fatty acids may involve the inhibition of the cardiac Na$^+$ channels and L-type Ca$^{2+}$ channels. The P450 epoxygenase metabolites of $\omega$-3 fatty acids (EEQs) are potent vasodilators in coronary microvessels (Zhang et al., 2001). It is intriguing that the $\omega$-3 fatty acid metabolite, 11,12-EEQ, is more potent than its $\omega$-6 epoxide homolog, 11,12-EET, in activating the cardiac K$_{ATP}$ channels. Whether this is related to the cardioprotective effects of $\omega$-3 fatty acids is not known, but the physiological function of $\omega$-3 fatty epoxides deserve further investigation. The $\omega$-3 double bond, undoubtedly renders the hydrocarbon tail more rigid and nucleophilic. This appears to be the only structural determinant studied that augmented the ability of the fatty epoxide to activate K$_{ATP}$ channels. Small changes in carbon chain length, lowered double bond numbers, altered epoxide positions, and the loss of the negative carboxylic charge did not affect the potency of the epoxide in reducing ATP sensitivity of the K$_{ATP}$ channels in cardiac myocytes.

The physiologic importance of K$_{ATP}$ channel activation by EET, in the presence of 5 mM ATP, is demonstrated by the hyperpolarization of resting membrane potentials and shortening of the action potentials. The effects of 11,12-EET were again stereospecific and completely reversed by glyburide, suggesting that these electrophysiological effects were mediated by the K$_{ATP}$ channels. Whether these effects are cardioprotective or not is at present unclear, although there appears to be a greater effect on membrane potential than on APD$_{90}$. Hyperpolarization of membrane potential would likely suppress abnormal automaticity and is therefore anti-arrhythmic in outcome. However, shortening of the action potential may promote reentry mechanisms of arrhythmia and may create dispersion of refractoriness, resulting in pro-arrhythmic effects. These issues will require detailed electrophysiological studies to resolve. Recently, blood in the coronary sinus of dog hearts showed significant increases in the levels of EETs and their metabolites during coronary occlusion and reperfusion (Nithipatikom et al., 2001). Under these conditions, EETs would accumulate in cardiomyocytes and ATP would be depleted, creating a situation in which significant activation of the K$_{ATP}$ channels could occur. Indeed, using a K$_{ATP}$ channel knockout mouse, the molecular basis of the ST segment elevation during acute myocardial infarction can be attributed to the activation of K$_{ATP}$ channels (Li et al., 2000).

In conclusion, we have identified the structural determinants of EET for activation of the cardiac K$_{ATP}$ channels and showed the effects of EET to be stereospecific. Our results indicate that EET can activate the K$_{ATP}$ channels under physiological conditions and may serve to regulate cardiac electrical excitability.


Address correspondence to: Dr. Hon-Chi Lee, Division of Cardiovascular Diseases, Department of Internal Medicine, Mayo Clinic, 200 First Street SW, Rochester, MN 55905. E-mail: lee.honchi@mayo.edu