Activation of rat mesenteric arterial $K_{ATP}$ channels by 11,12-epoxyeicosatrienoic acid

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Ye, Dan, Wei Zhou, and Hon-Chi Lee. Activation of rat mesenteric arterial $K_{ATP}$ channels by 11,12-epoxyeicosatrienoic acid. Am J Physiol Heart Circ Physiol 288: H358–H364, 2005. First published August 26, 2004; doi:10.1152/ajpheart.00423.2004.—Epoxyeicosatrienoic acids (EETs), the cytochrome P-450 epoxygenase metabolites of arachidonic acid, are candidates of endothelium-derived hyperpolarizing factors. We have previously reported that EETs are potent activators of cardiac ATP-sensitive $K^+$ ($K_{ATP}$) channels, but their effects on the vascular $K_{ATP}$ channels are unknown. With the use of whole cell patch-clamp techniques with 0.1 mM ATP in the pipette and holding at -60 mV, freshly isolated smooth muscle cells from rat mesenteric arteries had small glibenclamide-sensitive currents at baseline (13.1 ± 3.9 pA, n = 5) that showed a 7.2-fold activation by 10 $\mu$M pinacidil (94.1 ± 21.9 pA, n = 7, P < 0.05). 11,12-EET dose dependently activated the $K_{ATP}$ current with an apparent $EC_{50}$ of 87 nM. Activation of the $K_{ATP}$ channels by 500 nM 11,12-EET was inhibited by inclusion of the PKA inhibitor peptide (5 $\mu$M) but not by the inclusion of the PKC inhibitor peptide (100 $\mu$M) in the pipette solution. These results were corroborated by vasoreactivity studies. 11,12-EET produced dose-dependent vasorelaxation in isolated small mesenteric arteries, and this effect was reduced by 50% with glibenclamide (1 $\mu$M) preincubation. The 11,12-EET effects on vasorelaxation were also significantly attenuated by preincubation with cell-permeant PKA inhibitor myristoylated PKI(14–22), and, in the presence of PKA inhibitor, glibenclamide had no additional effects. These results suggest that 11,12-EET is a potent activator of the vascular $K_{ATP}$ channels, and its effects are dependent on PKA activities.

ATP-sensitive $K^+$ channel; mesenteric artery; protein kinase A

EPOXYEICOSATRIENOIC ACIDS (EETs), the cytochrome P-450 epoxygenase products of arachidonic acid, have emerged as important vasoreactive mediators (20, 38). EETs are highly potent vasodilators of the coronary and other vascular beds through activation of the large-conductance $Ca^{2+}$-activated $K^+$ (BKCa) channels, and are endothelium-derived hyperpolarizing factors (EDHFs) (4, 5, 23, 42). In addition, EETs have been shown to inhibit the cardiac Na+ channels (14), modulate cardiac L-type $Ca^{2+}$ channels (6, 34), inhibit the cloned human T-type $Ca^{2+}$ channel (39), and inhibit the tracheal Cl- channel (27). Recently, we found that EETs are potent activators of the cardiac ATP-sensitive $K^+$ ($K_{ATP}$) channels (17) with $EC_{50}$ values of 10–8 M. EETs activate cardiac $K_{ATP}$ channels directly, without the presence of second messengers, by reducing the channel sensitivity to ATP (17). EET effects are highly specific; neither arachidonic acid, its precursor, nor dihydroxyeicosatrienoic acid (its hydroxylase metabolite) had any effect on $K_{ATP}$ channel activation. Furthermore, the effects of EET are stereospecific, and the epoxide functional group is the critical structural determinant that is required for $K_{ATP}$ channel activation (19). However, the role of EET in modulating the vascular $K_{ATP}$ channels is unknown. In this study, we examined the effects of 11,12-EET on $K_{ATP}$ channel activities in isolated smooth muscle cells from small mesenteric arteries in the rat. We found that 11,12-EET potently activates the vascular $K_{ATP}$ channels, but its effects are mediated through PKA-dependent mechanisms. These results were further corroborated by vasoreactivity experiments showing that 11,12-EET produces potent vasorelaxation in isolated mesenteric arteries that are significantly inhibited by the $K_{ATP}$ channel inhibitor glibenclamide and by PKA inhibition. These findings suggest that EETs could be important endogenous vascular $K_{ATP}$ channel activators and may play an integral role in vascular tone regulation.

MATERIALS AND METHODS

Animals and isolation of arterial smooth muscle cells from rat small mesenteric arteries. The use of animals and the procedures involved with tissue isolation were approved by the Animal Care and Use Committee at the Mayo Clinic (Rochester, MN). Single vascular smooth muscle cells were prepared as previously described (36). Briefly, male Sprague-Dawley rats (200–250 g body wt) were anesthetized with pentobarbital (100 mg/kg ip). The bowels and mesenteries were rapidly excised and placed in Krebs solution, which contained (in mM) 118.3 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, 25 NaHCO3, and 11.1 dextrose. The third- and fourth-ordered branches (100–250 $\mu$m in intraluminal diameter) of mesenteric arteries were carefully dissected and isolated free of surrounding connective tissue under a dissection microscope. The small arteries were then placed in 1 ml of physiological saline solution containing (in mM) 145 NaCl, 4.0 KCl, 0.05 CaCl2, 1.0 MgCl2, 10.0 HEPES, and 10.0 glucose, pH 7.2 with 0.1% BSA at room temperature for 10 min, followed by treatment with 1.75 mg of papain (11.9 U/mg) and 1.25 mg of dithiothreitol in 1 ml of saline solution at 37°C for 10 min. The vessels were further digested with 1.25 mg of collagenase (CLS-2, 360 U/mg) and 1.25 mg of trypsin inhibitor in 1 ml of saline solution at 37°C for 10 min, washed three times with 1.0 ml aliquots of saline solutions, and then gently triturated with a fire-polished glass pipette until they were completely dissociated. The resulting smooth muscle cell suspension was stored at 4°C and used within 8 h.

Whole cell patch-clamp recordings. Whole cell currents were recorded from single smooth muscle cells using patch-clamp techniques as previously described (13, 14). Isolated smooth muscle cells were placed in a recording chamber on the stage of an inverted microscope with an extracellulat bath solution that contained (in mM) 134 NaCl, 6 KCl, 1 MgCl2, 0.1 CaCl2, 10 HEPES, and 10 glucose, pH 7.4. Cells were superfused with a 140 mM high-$K^+$ bath solution, made by substituting NaCl with KCl in the above solution, at 1–2 ml/min, and solution exchanges were complete within 30–60 s.

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Whole cell $K_{\text{ATP}}$ currents were recorded with a pipette solution that contained (in mM) 110 KCl, 30 KOH, 10 HEPES, 10 EGTA, 1 MgCl$_2$, 1 CaCl$_2$, 0.1 Na$_2$ATP, 0.1 Na$_2$ADP, and 0.5 Na$_2$GTP, pH 7.4, using an Axopatch 200 integrating amplifier (Axon Instruments; Foster City, CA), filtered at 1 kHz and sampled at 2 kHz. Pipette resistance ranged from 3 to 5 M$\Omega$, and seal resistance was >10 G$\Omega$. pCLAMP 8.0 software (Axon) was used for generating voltage-clamp protocols and for the acquisition and analysis of $K_{\text{ATP}}$ current. All cellular electrophysiology experiments were performed at room temperature (21–23°C). To minimize the activity of voltage-dependent K$^+$ (delayed rectifiers) channels, most of the experiments were performed at a negative holding potential of −60 mV and extracellular K$^+$ at 140 mM to elicit a substantial inward driving force for K$^+$ (13). To minimize the activity of BKCa channels, intracellular Ca$^{2+}$ was buffered by EGTA to low levels (~20 nM). In addition, 100 nM ibetorixin (IBTX) was present in the bath solution to block the BKCa channel activities that could be activated by EET (18). Glibenclamide-sensitive K$^+$ currents were determined and were referred to as $K_{\text{ATP}}$ currents.

**Vasoreactivity measurements.** Vasoreactivity of isolated small mesenteric arteries (100–250 $\mu$m in diameter) was determined by videomicroscopy as previously described (36, 40). Isolated small mesenteric arteries (~2 mm in length) were transferred to a vessel chamber filled with Krebs solution and were equilibrated for at least 30 min at 37°C before the start of the experiment. The arteries were mounted and secured between two borosilicate glass micropipettes (30-$\mu$m-diameter tips) with a 10-O ophthalmic suture. The lumen of the vessel was filled with Krebs solution through the micropipettes and maintained at a constant pressure (no flow) of 60 mmHg. The vessel chamber was transferred to the stage of an inverted microscope coupled to a video measurement system (VIA-100, Boeckeler Instruments), which was equipped with a video camera, monitor, and calibrated video calipers for visualization and recording of the intraluminal diameter. All compounds were added to the circulating bath, and the cumulative dose response was determined at 3- to 5-min intervals between doses. Vessels were constricted to about 40% of baseline diameter using endothelin-1. These vessels contained intact endothelium, and 10$^{-4}$ M acetylcholine produced 82.1 ± 4.7% vasorelaxation. The effects of 11,12-EET in relaxing these vessels were examined over the concentration range of 1 × 10$^{-10}$ to 1 × 10$^{-6}$ M. Vessels were discarded if they failed to produce an 85% relaxation with 1 × 10$^{-4}$ M sodium nitropusside or failed to produce a 30% constriction with 60 mM KCl.

**Materials.** 11,12-EET was obtained from Cayman Chemical (Ann Arbor, MI), solubilized in ethanol as a 5 mM stock, and stored at −80°C. The PKA inhibitor peptide myristoylated PKI(14–22) amide and the PKC inhibitor peptide myristoylated PKC(20–29) were obtained from BioMol (Plymouth Meeting, PA). Pinacidil, glibenclamide, dibutyryl cAMP, enzymes, and the rest of the chemicals were obtained from Sigma Chemical (St. Louis, MO). Pinacidil was solubilized in DMSO as a 10 mM stock. The rest of the chemicals were solubilized in water.

**Statistical analysis.** All data are expressed as means ± SE. A paired Student’s t-test was used to compare data obtained before and after intervention. One-way ANOVA was used to analyze data from multiple groups, and pairwise comparisons among the groups were performed using a post hoc Tukey key test. A statistically significant difference is defined as $P < 0.05$.

**RESULTS**

11,12-EET is a potent activator of the mesenteric $K_{\text{ATP}}$ channels. Mesenteric vascular smooth muscle cells exhibit very little glibenclamide-sensitive K$^+$ currents at baseline. With 0.1 mM ATP in the pipette, at a holding membrane potential of −60 mV and 140 mM extracellular K$^+$, the $K_{\text{ATP}}$ current amplitude was 13.1 ± 3.9 pA ($n = 5$). However, 10 μM pinacidil produced a 7.2-fold activation of these currents (94.1 ± 21.9 pA, $n = 7, P < 0.05$ vs. control; Fig. 1, A and B). In studying the effects of 11,12-EET on $K_{\text{ATP}}$ channels in vascular smooth muscle cells, we found that there was consistently an inward current being activated by 11,12-EET that would interfere with $K_{\text{ATP}}$ current measurements even at −60 mV (data not shown). This current could be blocked by 100 nM IBTX in the bath solution and represents the activated BKCa currents by 11,12-EET from a hyperpolarization shift in the current-voltage relationship (18). Hence, all experiments involving 11,12-EET were performed with 100 nM IBTX in the bath solution.

11,12-EET dose dependently activated $K_{\text{ATP}}$ currents in mesenteric smooth muscle cells. 11,12-EET (5 nM) had no effect (10.0 ± 1.0 pA, $n = 5$), but $K_{\text{ATP}}$ currents were increased by 44% with 50 nM 11,12-EET to 18.9 ± 2.6 pA ($n = 8$), by 181% with 500 nM 11,12-EET to 36.8 ± 6.2 pA ($n = 7, P < 0.05$ vs. control), and by 208% with 5 μM 11,12-EET to 40.4 ± 8.1 pA ($n = 7, P < 0.02$ vs. control; Fig. 1, C and D). The dose-response relationship fitted with a Hill equation showed an EC$_{50}$ of 87 nM and a Hill coefficient of 1.6 (Fig. 1D). These results indicate that 11,12-EET is a potent activator of mesenteric $K_{\text{ATP}}$ channels with an EC$_{50}$ in a similar concentration range to that for the cardiac $K_{\text{ATP}}$ channels (18). Although 11,12-EET is not as efficacious as pinacidil in $K_{\text{ATP}}$ channel activation, it is capable of producing 43% of the peak pinacidil effect.

The effects of 5 μM 11,12-EET on the current-voltage relationship of $K_{\text{ATP}}$ currents are shown in Fig. 2. In the presence of symmetrical K$^+$ at 140 mM and 100 nM IBTX in the bath, currents were elicited with a holding potential of 0 mV and pulsing from −100 to 40 mV in increments of 10 mV. The evoked currents were significantly activated by 11,12-EET, and the EET effects were blocked by glibenclamide (Fig. 2, A–C). Group results are shown in Fig. 2D, and 11,12-EET produced significant activation of $K_{\text{ATP}}$ currents at all negative potentials ($n = 7, P < 0.05$).

11,12-EET effects are mediated through PKA-dependent mechanisms. To further investigate the mechanism of $K_{\text{ATP}}$ channel activation by 11,12-EET, we examined the role of second messenger systems in mediating these effects. The $K_{\text{ATP}}$ channel is known to be modulated by protein kinases including PKA- and PKC-dependent mechanisms (10, 11, 13, 15, 26, 33, 37). Application of membrane-permeable dibutyryl cAMP at 0.5 mM resulted in a modest increase in $K_{\text{ATP}}$ currents and did not prevent subsequent current increase by 500 nM 11,12-EET (Fig. 3, A and B). However, exposure to 1 mM dibutyryl cAMP produced a much more robust $K_{\text{ATP}}$ current response, to levels similar to those induced by 500 nM 11,12-EET. Subsequent exposure to 500 nM 11,12-EET produced no further $K_{\text{ATP}}$ current increase (Fig. 3, C and D), suggesting that the stimulatory effects of 11,12-EET on $K_{\text{ATP}}$ channels might be cAMP mediated.

These results were confirmed by experiments with the PKA inhibitor peptide myristoylated PKI(14–22), included in the pipette solution for internal cellular dialysis. In the presence of 5 μM PKI(14–22), the ability of 500 nM 11,12-EET to activate $K_{\text{ATP}}$ currents was significantly attenuated, producing only 43 ± 30% of current increase (5.2 ± 2.3 pA increase, $n = 8$) compared with a 435 ± 116% of current increase under control conditions (31.5 ± 5.3 pA increase, $n = 7, P < 0.005$).
In contrast, with 100 μM PKC inhibitor peptide myristoylated PKC(20–28) in the pipette solution, no inhibition of the 11,12-EET effects was observed, with 870 ± 326% of KATP current increase (68.8 ± 22.8 pA increase, n = 4, P = not significant vs. control; Fig. 4). These results indicate that activation of mesenteric KATP channels by 11,12-EET is mediated through KATP channel activation. KATP currents were recorded at −60 mV showing pinacidil (Pin) activation of glibenclamide (Glib)-sensitive K⁺ currents. The dashed line indicates the zero current level. The vertical arrow here and in subsequent figures indicates when extracellular K⁺ was changed from 6 to 140 mM K⁺. Pinacidil (10 μM) and glibenclamide (10 μM) were added as indicated. B: group data comparing KATP (glibenclamide-sensitive) currents under control conditions when bath solution did not contain any drug (n = 5) and in the presence of 10 μM pinacidil (n = 7). *P < 0.05 vs. control. Control and pinacidil experiments were conducted in different cells. C: whole cell current recording showing the response to 5 μM 11,12-epoxyeicosatrienoic acid (EET). D: dose-response relationship of 11,12-EET on KATP channel activation. KATP currents were recorded at −60 mV as the glibenclamide-sensitive K⁺ current in the presence of various concentrations of 11,12-EET (5 × 10⁻⁸ M, n = 5; 5 × 10⁻⁹ M, n = 8; 5 × 10⁻¹⁰ M, n = 7; 5 × 10⁻¹¹ M, n = 7). Controls were superfused with buffer containing no EET (n = 5). Each cell was exposed to one dose of EET, and control cells were exposed to vehicle. Data are presented as means ± SE, and the continuous line represents the best fit by a Hill equation. The apparent EC₅₀ for 11,12-EET was 87 nM, and the Hill coefficient was 1.6.

All four EET regioisomers are effective activators of the KATP channels. To determine and compare the effects of the four EET regioisomers on KATP channel activation in vascular smooth muscle cells, we measured the increase in glibenclamide-sensitive K⁺ currents at −60 mV showing pinacidil (Pin) activation of glibenclamide (Glib)-sensitive K⁺ currents. The dashed line indicates the zero current level. The vertical arrow here and in subsequent figures indicates when extracellular K⁺ was changed from 6 to 140 mM K⁺. Pinacidil (10 μM) and glibenclamide (10 μM) were added as indicated. B: group data comparing KATP (glibenclamide-sensitive) currents under control conditions when bath solution did not contain any drug (n = 5) and in the presence of 10 μM pinacidil (n = 7). *P < 0.05 vs. control. Control and pinacidil experiments were conducted in different cells. C: whole cell current recording showing the response to 5 μM 11,12-epoxyeicosatrienoic acid (EET). D: dose-response relationship of 11,12-EET on KATP channel activation. KATP currents were recorded at −60 mV as the glibenclamide-sensitive K⁺ current in the presence of various concentrations of 11,12-EET (5 × 10⁻⁸ M, n = 5; 5 × 10⁻⁹ M, n = 8; 5 × 10⁻¹⁰ M, n = 7; 5 × 10⁻¹¹ M, n = 7). Controls were superfused with buffer containing no EET (n = 5). Each cell was exposed to one dose of EET, and control cells were exposed to vehicle. Data are presented as means ± SE, and the continuous line represents the best fit by a Hill equation. The apparent EC₅₀ for 11,12-EET was 87 nM, and the Hill coefficient was 1.6.

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Vasorelaxation of small mesenteric arteries by 11,12-EET is mediated through KATP channel activation. To further determine the physiological relevance of vascular KATP channel activation by EET, we examined the ionic mechanisms of mesenteric vasorelaxation by 11,12-EET. 11,12-EET produced potent dose-dependent vasorelaxation of small mesenteric arteries with 3.8 ± 0.3% relaxation at 10⁻¹⁰ M, 10.1 ± 1.6% relaxation at 10⁻⁹ M, 22.2 ± 2.3% relaxation at 10⁻⁸ M, 37.2 ± 2.6% relaxation at 10⁻⁷ M, and 49.4 ± 2.6% relaxation at 10⁻⁶ M (n = 5). These values were similar to those reported in rat mesenteric arteries (22) and in bovine coronary arteries (4). After incubation with 1 μM glibenclamide for 30 min, the vasorelaxation effects of 11,12-EET in small mesenteric arteries were significantly attenuated at all doses, with only 10.6 ± 2.0% relaxation at 10⁻⁸ M and 27.1 ± 2.3% relaxation at 10⁻⁶ M (n = 6, P < 0.05 vs. control for all doses) (Fig. 6). These results indicate that one-half of the EET vasorelaxation effects in rat small mesenteric arteries are mediated through activation of KATP channels. After incubation with 100 nM IBTX for 30
min, the vasorelaxation effects of 11,12-EET were also significantly attenuated at all doses, with only 7.0 ± 1.4% relaxation at 10⁻⁸ M and 23.8 ± 1.8% relaxation at 10⁻⁶ M (n = 5, P < 0.05 vs. control for all doses), suggesting that the BKCa channels also contribute significantly to EET-mediated vasorelaxation in these vessels. Preincubation with both IBTX and glibenclamide had an additive effect, resulting in almost complete inhibition of EET vasorelaxation effects (Fig. 6).

To determine whether EET-mediated vasorelaxation is dependent on PKA activity, we preincubated isolated mesenteric vessels with cell-permeable myristoylated PKI(14–22) (5 μM) for 45 min and found that the vasodilatory effects of 11,12-EET were significantly attenuated at all doses, with 8.8 ± 1.2% relaxation at 10⁻⁸ M and 28.9 ± 2.3% relaxation at 10⁻⁶ M (n = 6, P < 0.05 vs. control at all doses). Preincubation with both PKA inhibitor and glibenclamide had no additional dim-

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Fig. 2. Effect of 11,12-EET on the current-voltage (I-V) relationship of KATP currents. Whole cell currents were elicited in the presence of symmetrical 140 mM K⁺ and 100 nM iberiotoxin (IBTX) from a holding potential of 0 mV to testing pulses of −100 to +40 mV in 10-mV increments. Recordings from a typical cell show currents at baseline (A), with 5 μM 11,12-EET (B), and with 5 μM 11,12-EET + 10 μM glibenclamide (C). D: I-V relationship of KATP (glibenclamide sensitive) currents at baseline and with 5 μM 11,12-EET (n = 7, *P < 0.05 vs. baseline).

Fig. 3. Activation of mesenteric KATP currents by dibutyryl cAMP (db-cAMP). A: whole cell current recording showing minimal current activation by 0.5 mM dibutyryl cAMP, whereas subsequent exposure to 500 nM 11,12-EET resulted in significant activation of glibenclamide-sensitive (10 μM) currents. B: group data showing the KATP current at baseline, after exposure to 0.5 mM db-cAMP, and after exposure to 0.5 mM db-cAMP + 500 nM 11,12-EET (n = 7, *P < 0.05 vs. baseline and **P < 0.005 vs. baseline). C: whole cell K⁺ current recording showing activation by 1 mM db-cAMP and subsequent exposure to 500 nM 11,12-EET showed no further current activation. D: group data showing the KATP current densities at baseline, after exposure to 1 mM db-cAMP, and after exposure to 1 mM db-cAMP + 500 nM 11,12-EET (n = 7, **P < 0.005 vs. baseline).
activation of its vasorelaxation effects.

Activating mesenteric KATP channels by 11,12-EET is mediated through PKA signaling cascade, which is not dependent on any cytosolic second messengers, activation of mesenteric KATP channels by 11,12-EET is mediated through PKA-

Fig. 4. 11,12-EET activation of K<sub>ATP</sub> channels is inhibited by PKA inhibitor (PKI) but not by PKC inhibitory peptide (PKC-IP). Whole cell current recordings with the pipette solution containing 5 μM PKI and exposure to 500 nM 11,12-EET failed to activate the K<sub>ATP</sub> current (A), and at 100 μM PKC-IP and 500 nM 11,12-EET activation. K<sub>ATP</sub> currents remained intact (B). C: group data showing the activation of K<sub>ATP</sub> currents by 500 nM 11,12-EET with no inhibitor peptide (control, n = 7), with 5 μM PKI (n = 8), and with 100 μM PKC-IP (n = 4) in the pipette solution. *P < 0.05 vs. control.

The major findings in this study include the following. First, 11,12-EET is a potent activator of mesenteric K<sub>ATP</sub> channels, with an EC<sub>50</sub> of 87 nM, similar to that reported in cardiac K<sub>ATP</sub> channels. Second, all four EET regioisomers are effective activators of the vascular K<sub>ATP</sub> channels. Third, in contrast to EET activation of cardiac K<sub>ATP</sub> channels, which does not require any cytosolic second messengers, activation of mesenteric K<sub>ATP</sub> channels by 11,12-EET is mediated through PKA-dependent mechanisms. Finally, activation of K<sub>ATP</sub> channels in small mesenteric arteries by EET is a physiologically important mechanism, accounting for 50% of the EET vasorelaxation effects.

K<sub>ATP</sub> channels are ubiquitous and play a unique role in coupling the cellular metabolic state to excitability (28, 30, 37). Cardiac K<sub>ATP</sub> channels have been shown to modulate the cardiac action potential and contractility (31) and protect the heart against ischemia and stress (32, 41). Vascular K<sub>ATP</sub> channels are thought to play important roles in the vascular

Fig. 5. Effect of EET regioisomers and isoproterenol (Iso) on K<sub>ATP</sub> current activation. Bar graphs represent the glibenclamide-sensitive currents elicited by a voltage step from 0 to −100 mV in the presence of vehicle (control, n = 5), 5 μM 5,6-EET (n = 5), 5 μM 8,9-EET (n = 5), 5 μM 11,12-EET (n = 7), 5 μM 14,15-EET (n = 5), and 10 μM Iso (n = 5). Extra- and intracellular K+ were symmetrical at 140 mM. *P < 0.05 vs. control.

Previous studies from different laboratories have shown that EETs function as EDHFs in the coronary and other circulations (4, 5, 8). EETs are potent activators of vascular BK<sub>Ca</sub> channels, producing membrane hyperpolarization, leading to vasorelaxation (4, 23, 40). The role of EETs on vascular K<sub>ATP</sub> channel activation has not been studied directly, although Fukao et al. (9) reported that 11,12-EET-induced smooth muscle hyperpolarization. The role of EETs on vascular K<sub>ATP</sub> channel activation has not been studied directly, although Fukao et al. (9) reported that 11,12-EET-induced smooth muscle hyperpolarization.

Fig. 6. Dose-dependent vasorelaxation of small mesenteric arteries by 11,12-EET is glibenclamide and PKI sensitive. Isolated mesenteric arteries were constricted to about 40% baseline diameter with endothelin-1, and the vasorelaxation effect of various concentrations of 11,12-EET (1 × 10<sup>−10</sup> to 1 × 10<sup>−8</sup> M) was determined after incubation with no drug (control), with 1 μM glibenclamide, with 5 μM PKI, with 5 μM PKI + 1 μM glibenclamide, with 100 nM IBTX, and with 1 μM glibenclamide + 100 nM IBTX. Data are presented as means ± SE; n = 5 for control and IBTX and n = 6 for other groups. *P < 0.05 vs. control; **P < 0.05 vs. IBTX.
larization in isolated rat mesenteric arteries was blocked by glibenclamide, suggesting that K_{ATP} channels were involved. We have recently reported that EETs are potent activators of cardiac K_{ATP} channels (17, 19). EETs markedly reduce the channel sensitivity to ATP, allowing these channels to open even in the presence of physiological concentrations of intracellular ATP (mM range). EETs increase K_{ATP} channel opening probability, increase channel open dwell times, reduce channel closed times, and eliminate the log closed state, leading to significant hyperpolarization of resting membrane potential in cardiac myocytes (17). In addition, the effects of EETs are stereospecific; only the 11(S),12(R)-EET enantiomer could activate cardiac K_{ATP} channels and produce resting potential hyperpolarization, with the 11(R),12(S)-EET enantiomer being totally inactive (19). These observations were made in inside-out patches, suggesting a direct EET effect on the channel without the requirement of diffusible second messengers. In the present study, we found that activation of mesenteric K_{ATP} channels by 11,12-EET is mediated by a PKA-dependent mechanism, underscoring the differences in pharmacological regulation between the cardiac and vascular channel isoforms. Indeed, the functional cardiac K_{ATP} channel is encoded by the SUR2A and Kir6.2 subunits, whereas the vascular channel is formed by the coupling of SUR2B and Kir6.1 subunits, and they have inherent differences in channel unitary conductance as well as in the sensitivity to nucleotide diphosphates and to ATP (1, 26, 29, 37).

PKA has not been found to be an obligatory step in mediating the EET regulation of ion channels. Activation of BK_{Ca} channels by EETs in vascular smooth muscles is not cAMP dependent (4), and cardiac Na+ channels are inhibited by EETs through a “modulated receptor” mechanism that is not cAMP mediated (14). However, EETs have been shown to activate cardiac L-type Ca2+ channels through cAMP-dependent mechanisms (34), although the mechanism through which EETs produce an increase in cAMP level is not clear, and evidence supporting the presence of a definitive plasmalemmal EET receptor has been elusive. With the use of the N-methylsulfonylimide analog of 11,12-EET, which resists esterification and metabolite formation in vascular smooth muscle cells, producing membrane hyperpolarization and vasorelaxation.

Activities of the K_{ATP} channels have been shown to be modulated by PKA- and PKC-mediated phosphorylation (10, 11, 13, 15, 26, 37). The effects of PKC appear to be stimulatory on cardiac K_{ATP} channels (11, 15) but inhibitory on vascular K_{ATP} channels (7, 10, 13). In contrast, the effects of PKA on K_{ATP} channels have been uniformly stimulatory (16, 37). Our observations that 11,12-EET activates mesenteric K_{ATP} channels with an EC_{50} of 87 nM and that 500 nM 11,12-EET is capable of producing 43% of the peak effect of 10 μM pinacidil indicate that 11,12-EET could be an important endogenous regulator of vascular K_{ATP} channel activity. This mechanism of vasodilation should become even more important during ischemia or hypoxia when production of EET is enhanced and intracellular ATP is depleted (25). Indeed, overexpression of cyttochrome P-450 CYP2J2 has been shown to protect against hypoxia-reoxygenation injury in cultured endo-

thelial cells (35), and neuroprotection by experimental transient ischemic attacks is linked to the upregulation of cytochrome P-450 2C11 (2). In addition, Kir6.2 knockout mice have lost the cardioprotection effects of ischemic preconditioning (32). Recently, hypoxia-induced vasodilation in human coronary arterioles mediated by K_{ATP} opening was found to be impaired in diabetes (24). Such findings suggest that activation of vascular K_{ATP} channels by EETs could serve as an important protective mechanism for the cardiovascular system against ischemic and metabolic insults.

GRANTS

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REFERENCES


