EPOXYEICOSATRIENOIC ACID ENHANCES Cl^- CURRENTS IN CULTURED SHARK RECTAL GLAND CELLS

Qingen Ke[1], Martha Ratner[2], Yinke Yang[1], John N. Forrest, Jr.[2] and Yong-Fu Xiao[1]

[1]Cardiovascular Division, Department of Medicine, Beth Israel Deaconess Medical Center Harvard Medical School, Boston, MA 02215
[2]Department of Medicine, Yale University School of Medicine, New Haven, CT 06510

Cytochrome P450 (P450) catalyzes a wide variety of endogenous and exogenous compounds. The earliest P450 in evolution probably participated in the synthesis and degradation of fatty acids and steroids. Detoxification of foreign substance by P450 came later. P450 has been detected in all living systems examined. Cyclooxygenase, lipoxygenases, and cytochrome P450-dependent monooxygenases are three major enzyme systems for the oxidative metabolism of AA, arachidonic acid (McGiff, *Ann. Rev. Pharmacol. Toxicol.* 31:339-369, 1991). The third pathway generates a variety of AA metabolites, including four epoxide regioisomers (5,6-, 8,9-, 11,12-, and 14,15-epoxyeicosatrienoic acids, EETs). These AA products catalyzed by P450 have been shown to affect ion channel activities (Hu and Kim, *Eur. J. Pharmacol.* 230:215-221, 1993; Xiao et al., *J. Physiol. Lond.* 508:777-792, 1998; Yuan et al., *Am.J. Physiol.* 268:C259-C270, 1995). Cyclic AMP-activated Cl^- channels of shark rectal gland (SRG) cells are critical for the secretion of salt and water in the spiny dogfish, *Squalus acanthias*. This study was designed to examine 11,12-EET, one of the P450-mediated products of AA, on Cl^- currents in primary cultured SRG cells.

Enzymatically isolated single SRG cells were plated on glass coverslips and cultured for 6 to 10 days until they were used for experiments (Valentich & Forrest, Am. J. Physiol. 260:C813-C823, 1991). Whole-cell Cl^- currents were recorded as the method described previously (Xiao, Life Sci. 60:2231-2243, 1997). The pipette solution contained (in mM) 240 CsCl or KCl, 20 Hepes, 0.2 EGTA, 5 MgATP, 1 $MgCl_2$, 70 TMAO, 300 urea, and pH 7.2. The bath solution contained (in mM) 270 NaCl, 250 urea, 5 CsCl, 5 $CaCl_2$, 20 Hepes, 3 $MgCl_2$, 1 glucose, and pH 7.2. Compounds used in this study were obtained from Sigma (St. Louis, MO), except 11,12-EET (Cayman Chemical Co., Ann Arbor, MI). External solutions were exchanged with a rapid perfusion system. Experiments were carried out at 21-23^{0}C. Statistical difference was examined by the Student's *t*-test and $p < 0.05$ was considered significantly different.

Extracellular perfusion of 11,12-EET solution at 40 nM produced a gradual increase in the whole-cell Cl^- current (I_{Cl}) in cultured SRG cells. The increase initiated around 3 min and reached the maximal effect about 8 to 10 min after 11,12-EET application. Fig. 1A shows that the voltage-dependent Cl^- currents were markedly increased after 10-min application of 11,12-EET at 40 nM (11,12-EET). EET-induced increase was washable and I_{Cl} returned to control levels after 8-min washout with the EET-free bath solution (Fig. 1A, Washout). Fig. 2B shows the current-voltage activation of whole-cell Cl^- currents (n = 11) in the absence (○, control; ▲, Washout) and presence (●) of 40 nM 11,12-EET.

The increase in I_{Cl} by 11,12-EET was concentration-dependent. Fig. 2 shows that the average currents were significantly increased, from –141 ± 18 pA (mean ± SEM) for control to –250 ± 25 pA ($n = 5$, $p < 0.01$) for 4 nM and to -1041 ± 25 pA for 40 nM ($n = 11$, $p < 0.001$) EET, respectively. The current was returned to –174 ± 25 pA after 8- to 10-min washout of 11,12-EET. The difference between control and washout was not significant ($p > 0.05$).

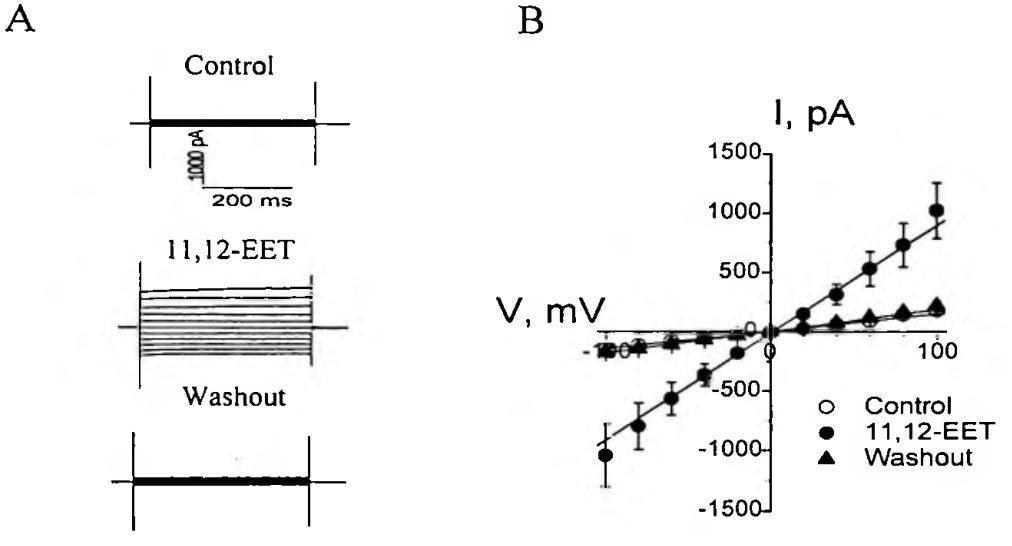

Figure 1. Effect of 11,12-EET on Cl^- currents in primary cultured SRG cells. **A**. Voltage-dependent current traces were elicited in the absence (Control & Washout) and presence of 40 nM 11,12-EET (EET). The membrane potential was held at 0 mV and currents were activated by 400-ms pulses from -100 to 100 mV in 20-mV increments every 5 s. **B**. Current-voltage relationship curves of I_{Cl} ($n = 11$) are shown for control (○), 40 nM 11,12-EET (●), and washout of EET (▲). The amplitude of currents was measured at 200-ms place of each current trace.

EETs have been shown to activate K^+ channels in vascular smooth muscle cells (Hu and Kim, *Eur. J. Pharmacol.* 230:215-221, 1993; Yuan et al., *Am.J. Physiol.* 268:C259-C270, 1995) and to affect vascular tone (Carroll et al., *Eur. J. Pharmacol.* 138:281-283, 1987; Harder et al., *Am. J. Physiol.* 266:H2098-H2107, 1994; Proctor et al., *Circ. Res.* 60:50-59, 1987). Importantly, P450 and AA metabolites may play important roles in development of some diseases. For example, EETs in the kidney act as a key factor for development of spontaneous or salt-induced hypertension in rats (Basu et al., *Hypertension* 24:480-485, 1994; Da Silva et al., *Am. J. Med. Sci.* 307:173-181, 1994). In addition, urinary excretion of epoxygenase metabolites is increased during pregnancy-induced hypertension in humans (Catella et al., *PNAS USA* 87:5893-5897, 1990). Therefore, EETs may play important roles for renal functions. In the present study 11,12-EET increased Cl^- currents in cultured SRG cells. Activation of Cl^- channels in SRG cells requires cAMP-dependent phosphorylation of the channel protein. The cellular mechanism of EET-enhanced Cl- currents in SRG cells is unclear, but G-protein (G_s) was involved in EET-induced an increase in K^+ currents in coronary smooth muscle cells (Li and Campbell, *Circ.*

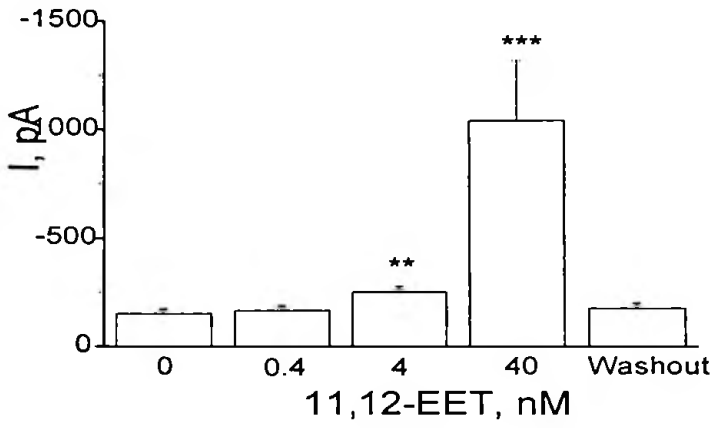

Figure 2. Concentration-dependent increase in Cl^- currents in cultured SRG cells. The membrane potential was held at 0 mV. Cl^- currents were activated by 400-ms pulses from a holding potential of 0 mV to -100 mV every 5 s. The amplitude of currents was measured at 200-ms place of each current trace. The average values of I_{Cl} are shown for control (n = 16), 0.2 nM (n = 5), 4 nM (n = 5), and 40 nM (n = 11) of 11,12-EET. The data were collected after 8- to 10-min application of EET. The values for washout were measured after 10-min washout of EET. **, $p < 0.01$; ***, $p < 0.001$; vs. control.

Res. 80:877-884, 1997). In addition, in isolated single cardiomyocytes 11,12-EET enhanced L-type Ca^{2+} currents accompanied with an increase in intracellular cAMP content (Xiao et al., *J. Physiol. Lond.*. 508:777-792, 1998). Therefore, 11,12-EET may directly or indirectly stimulate the adenylyl cyclase and(or) inhibit phosphodiesterase to raise intracellular cAMP levels. This increase in cAMP-production may in turn enhance phosphorylation of Cl^- channel protein and increase Cl^- currents in cultured SRG cells. Further experiments are required to elucidate the mechanism of EET-induced activation of Cl^- currents in SRG cells.

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