Inhibition of Soluble Epoxide Hydrolase by *trans*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid Is Protective Against Ischemia–Reperfusion Injury

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Abstract: Arachidonic acid, a polyunsaturated fatty acid, can be metabolized to cardioprotective epoxyeicosatrienoic acids (EETs) by cytochrome P450 epoxygenases, which are subsequently hydrolyzed to less bioactive dihydroxyeicosatrienoic acids by soluble epoxide hydrolase (sEH). To study the effects of pharmacological inhibitor of sEH (sEHi), C57BL6 mice hearts were perfused in Langendorff mode for 40 minutes of baseline and subjected to 30 minutes of global no-flow ischemia followed by 40 minutes of reperfusion. Hearts were perfused with the sEHi, *trans*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (*t*-AUCB; 0.05, 0.1, 0.5, and 1 μM). To study the mechanism(s), hearts were perfused with 0.1 μM *t*-AUCB in the presence or absence of putative EET receptor antagonist 14,15-epoxyeicosa-5(Z)-enoic acid (10 μM) or phosphatidylinositol 3-kinase (PI3K) inhibitors wortmannin (200 nM) or LY294002 (5 μM). Infarct size was determined at the end of 2-hour reperfusion by 2,3,5-triphenyltetrazolium chloride staining. Inhibition of sEH by *t*-AUCB significantly improved postischemic left ventricular developed pressure (LVDP) recovery and reduced the infarct size after ischemia and reperfusion, as compared with control hearts. Perfusion with 14,15-epoxyeicosa-5(Z)-enoic acid, wortmannin or LY294002 before ischemia abolished the cardioprotective phenotype; however, co-perfusion of both *t*-AUCB and 11,12-EET did not result in an additive effect on improved LVDP recovery. Together, our data suggest that pharmacological inhibition of sEH by *t*-AUCB is cardioprotective.

Key Words: sEH inhibitors, ischemia and reperfusion, cardioprotection, epoxyeicosatrienoic acid

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INTRODUCTION

Arachidonic acid is a polyunsaturated fatty acid that is present in the phospholipids of cell membranes, which can be released into cytosol in response to stressors such as ischemia.[1,2] Released free arachidonic acid can then be metabolized by cyclooxygenases, lipoxygenases, and cytochrome P450 monooxygenases.[1,3] Biologically active regioisomers, epoxyeicosatrienoic acids (5,6-, 8,9-, 11,12-, and 14,15-EET), are products by cytochrome P450 monooxygenases.[2,3] EETs can be reincorporated into phospholipid membranes or metabolized to smaller reactive epoxides by β-oxidation.[4,5] However, the predominant pathway of EET metabolism is conversion to the less active vicinal diols, dihydroxyeicosatrienoic acids (5,6-, 8,9-, 11,12-, and 14,15-DHET), by soluble epoxide hydrolases (sEH).[4,5]

EETs act as important cellular lipid mediators in the cardiovascular, renal, and nervous systems.[3,6–8] EETs have been shown to have protective effects against ischemia–reperfusion injury.[1,9–14] Our current understanding of the cardioprotective mechanisms of EETs suggests involvement of signaling pathways including phosphoinositide 3-kinase (PI3K)–Akt, increased secretion of cardiac hormones, and activation of cardiac ion channels such as adenosine triphosphate–sensitive K^+ channels.[1,9,10,12,13] Recent evidence indicates that PI3K/Akt or natriuretic peptide pathways activated by EETs converge onto the mitochondria, thereby limiting mitochondrial damage from ischemia–reperfusion injury.[10,12]

Previously, we demonstrated that targeted deletion of sEH gene is protective against ischemia–reperfusion injury.[1,10] Recently, Motoki et al[15] has reported pharmacological inhibition of sEH with 12-(3-adamantan-1-yl-ureido)-dodecanoic acid *n*-butyl ester (AUDA-nBE) was cardioprotective. In the present study, we report cardioprotective effects of more potent water-soluble and metabolically stable sEH inhibitor (sEHi), *trans*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (*t*-AUCB), than AUDA-nBE. Moreover, our data demonstrate marked reduction in infarction and improved contractile function at nanomolar concentrations, which involved the PI3K pathway.

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B. D. Hammock founded Arete Therapeutics to move sEH inhibitors to the clinic. S. H. Hwang and B. D. Hammock are authors of University of California patents in the area.

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MATERIALS AND METHODS

Animals

All experiments used male and female mice aged 3–5 months weighing 22–33 g and were treated in accordance with the guidelines of Health Science Laboratory Animal Services (HSLAS), University of Alberta. C57BL6 mice were purchased from Charles River Laboratories (Pointe Claire, PQ). A colony of mice with targeted disruption of the *Ephx2* gene (sEH null) and backcrossed onto a C57BL6 genetic background for more than 7 additional generations is maintained at the University of Alberta.

Chemicals

EETs were a kind gift from Dr J. R. Falck (University of Texas Southwestern Medical Center, Dallas, TX). Stock solutions (5 mM) were dissolved in 100% ethanol and working solutions were diluted in perfusion buffer (1 μM). The sEHi, *t*-AUCB, was synthesized in the laboratory of Dr. Bruce Hammock (UC Davis) and dissolved in dimethyl sulfoxide to make 10 mM stock solution. The putative EET receptor antagonist, 14,15-epoxyeicosa-5(Z)-enoic acid (14,15-EEZE) was a kind gift from Dr J. R. Falck and dissolved in 100% ethanol to make 5 mM stock solution. PI3K inhibitors, Wortmannin (Sigma-Aldrich, Oakville, ON) and LY294002 (Cell Signaling Technology, Inc, Danvers, MA), were dissolved in DMSO to make 5 and 50 mM stock solutions, respectively.

Isolated Heart Perfusions

Hearts were perfused in the Langendorff mode as previously published.[1,10] Briefly, hearts from age-/sex-matched mice were perfused in a retrograde fashion at constant pressure (90 cmH_2O) with continuously aerated ($95\%O_2/5\%CO_2$) Krebs–Henseleit buffer at 37°C. Hearts were perfused with buffer for 40 minutes of stabilization period and then subjected to 30-minute global no-flow ischemia, followed by 40-minute reperfusion. To determine *t*-AUCB dose and response, hearts from C57Bl6 mice were perfused with increasing concentrations of *t*-AUCB (0, 0.05, 0.1, 0.5, or 1 μM). For some experiments, hearts were perfused for 40-minute baseline, subjected to 30-minute ischemia, and then perfused with 0.1 μM *t*-AUCB in the presence or absence of putative EET receptor antagonist 14,15-EEZE (10 μM) and PI3K inhibitors wortmannin (200 nM) or LY294002 (5 μM). The percentage of left ventricular developed pressure (%LVDP) at 40 minutes of reperfusion (R40), as compared with baseline LVDP, was taken as a marker for recovery of contractile function. After 40 minutes of reperfusion, hearts were immediately frozen and stored below −20°C.

Infarct Size Analysis

To determine the amount of infarction, after 40 minutes of stabilization period and 30 minutes global no-flow ischemia, hearts were reperfused for 2 hours. After 2-hour reperfusion, hearts were perfused with 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC) dissolved in Krebs–Henseleit buffer at 37°C for 10 minutes, then fixed in formalin, and cut into thin cross-sectional slices. The area of infarction was quantified by measuring stained (red, live tissue) and unstained (white, necrotic) regions using Image J (National Institutes of Health, Bethesda, MD).

Statistical Analysis

Values expressed as mean ± standard error of mean. Statistical significance was determined by the unpaired Student *t* test and one-way analysis of variance followed by Newman–Keuls and Duncan tests to assess differences between groups. Values were considered significant if $P < 0.05$.

RESULTS

Cardioprotective Effects of *t*-AUCB

Recent evidence suggests that sEH is a good target in the prevention of ischemia–reperfusion injury.[1,10,15] To examine the effects of pharmacological inhibition of sEH on ischemia–reperfusion injury, we first performed a dose–response study. We perfused the wild type mouse hearts with 0, 0.05, 0.1, 0.5, and 1 μM of *t*-AUCB and monitored LVDP for postischemic functional recovery. No significant differences in baseline contractile function were observed between the groups, except LVDP in 1 μM *t*-AUCB–treated hearts, during aerobic baseline perfusion (Table 1). Hearts perfused with *t*-AUCB had significantly improved postischemic recovery of LVDP compared with control mice (Fig. 1B). The improved postischemic recovery followed a bell-shaped response, with the most improved functional recovery occurring at the mid-dose (0.1 μM). Therefore, 0.1μM *t*-AUCB was used to further study the cardioprotective effects.

To further assess the cardioprotective effects of *t*-AUCB, we analyzed the infarct size after 2-hour reperfusion in *t*-AUCB–treated hearts and vehicle control hearts. Significant decrease in infarct size was observed in the hearts treated with 0.1 μM *t*-AUCB compared with vehicle controls (Fig. 1C). Consistent with 40-minute reperfusion, postischemic functional recovery remained significantly higher at 2-hour reperfusion in *t*-AUCB–treated hearts compared with vehicle controls (LVDP = 43.3% ± 13.3% vs. 21.2% ± 1.8%).

Pharmacological or Genetic Inhibition of sEH and Cardioprotection

To compare the cardioprotective effects of genetic and pharmacological inhibition of sEH, we perfused sEH null mice with vehicle and WT mice with 0.1μM *t*-AUCB. Significant increase in postischemic functional recovery of sEH null (LVDP = 47.3% ± 6.2%) and *t*-AUCB perfused WT (LVDP = 48.0% ± 7.2%) was observed after ischemia–reperfusion protocol, compared with vehicle controls (LVDP = 22.4% ± 1.7%) and WT (LVDP = 19.7% ± 3.8%) (Fig. 2A, Table 2). No significant differences were observed in cardiac parameters during baseline (Table 2). Consistent with LVDP, rate of contraction and relaxation were significantly higher in sEH null hearts and *t*-AUCB–treated hearts after ischemia and reperfusion (Table 2). No significant differences were observed in heart rate during baseline or after ischemia and reperfusion (Table 2). The improved function was evident within 30 minutes of reperfusion after treatment with 0.1 μM *t*-AUCB and sEH null mice, which persisted throughout the recovery period (Fig. 2B).

TABLE 1. Cardiac Parameters for *t*-AUCB Dose Response

	Vehicle Control (n = 11)	0.05 μM *t*-AUCB (n = 4)	0.1 μM *t*-AUCB (n = 6)	0.5 μM *t*-AUCB (n = 5)	1 μM *t*-AUCB (n = 4)
Isolated perfused heart					
Preischemic					
LVDP (cmH_2O) (baseline)	112.7 ± 7.9	126.9 ± 13.2	103.9 ± 5.7	132.4 ± 16.1	168.4 ± 12.5*
Rate of contraction, dP/dt_{max} (cmH_2O/msec) (baseline)	3249 ± 294	5021 ± 318	3223 ± 213	3757 ± 517	4089 ± 451
Rate of relaxation, $-dP/dt_{min}$ (cmH_2O/msec) (baseline)	−2683 ± 188	−3926 ± 225	−2670 ± 168	−3054 ± 398	−3688 ± 333
HR, perfused (beats/min) (baseline)	291 ± 19	334 ± 13	355 ± 19	277 ± 46	268 ± 21
Postischemic					
LVDP (cmH_2O) (R40)	25.1 ± 2.9	52.0 ± 5.6*	47.0 ± 8.8*	57.2 ± 14.7*	51.8 ± 9.4*
Rate of contraction, dP/dt_{max} (cmH_2O/msec) (R40)	807 ± 92	1582 ± 115*	1404 ± 289*	1697 ± 415*	1477 ± 275*
Rate of relaxation, $-dP/dt_{min}$ (cmH_2O/msec) (R40)	−688 ± 82	−1370 ± 143*	−1326 ± 169*	−1367 ± 320*	−1285 ± 203*
HR, perfused (beats/min) (R40)	323 ± 16	337 ± 25	340 ± 16	347 ± 21	298 ± 27

Hemodynamic parameters were measured in isolated-perfused hearts. Values represent mean ± standard error of mean.
*$P < 0.05$ versus vehicle control.
HR, heart rate; R40, 40 minutes of reperfusion.

EET-Mediated Effects of *t*-AUCB

As previously reported, targeted deletion or pharmacological inhibition of sEH leads to increase in intracellular EET levels, which can further produce cardioprotective effects after ischemia–reperfusion injury.[1] To confirm the EET-mediated effects of *t*-AUCB, we perfused the WT hearts with 0.1 μM *t*-AUCB in presence or absence of putative pan-EET receptor antagonist 14,15-EEZE (10 μM). 14,15-EEZE did not affect the baseline function of the WT mice hearts (data not shown). However, postischemic functional recovery of *t*-AUCB–treated hearts (LVDP = 49.9% ± 7.5%) was completely abolished in the hearts coperfused with 14,15-EEZE (LVDP = 13.3% ± 2.5%) (Fig. 3A). To assess additive effects of exogenous EETs on the *t*-AUCB cardioprotective response, we perfused the WT hearts with *t*-AUCB in presence or absence of 11,12-EET (1 μM) and 14,15-EET (1 μM). Interestingly, no additive effects of exogenous EET were observed on *t*-AUCB–treated hearts (Fig. 3B). These data suggests that maximum recovery was achieved by treating the hearts with 0.1 μM *t*-AUCB, and therefore, addition of exogenous EETs do not show any additional effect on functional recovery.

PI3K-Mediated Effects of *t*-AUCB

To determine the role of the PI3K signaling cascade in *t*-AUCB-mediated cardioprotective response, we performed isolated perfused heart experiments in the presence or absence of the PI3K inhibitors wortmannin or LY294002. Neither inhibitor had a significant effect on baseline LVDP (data not shown). Perfusion with either wortmannin or LY294002 for 5 minutes before ischemia had no effect on postischemic LVDP recovery in vehicle controls; however, both inhibitors significantly reduced the improved postischemic functional recovery in *t*-AUCB–treated hearts (Fig. 4). Thus, percent LVDP recovery at 40-minute reperfusion was comparable in both the groups after treatment with either wortmannin or LY294002 (Fig. 4). These data suggest the involvement of the PI3K cascade in the cardioprotective effects of pharmacological inhibition of sEH.

DISCUSSION

In this article, we report improved postischemic contractile function and reduced infarct size in isolated mice hearts perfused with a novel water-soluble and potent pharmacological sEHi, *t*-AUCB. The effects of *t*-AUCB were attenuated by the putative EET-receptor antagonist 14,15-EEZE and inhibition of PI3K. Taken together, our data suggest that pharmacological inhibition of sEH by *t*-AUCB can be protective against ischemia–reperfusion injury and the effects are mediated through EETs and PI3K pathway.

sEH is a bifunctional enzyme with C-terminus hydrolase activity and N-terminus phosphatase activity.[5,16] Although the functional effects of phosphatase are not well known, much evidence implicates the importance of its hydrolase activity in cardiovascular diseases.[1,5,11,15,17,18] Two different approaches have been used to study the effects of sEH on cardiovascular system: genetic modification and pharmacological inhibition.[1,11,15] Genetic modulation by targeted deletion of *Ephx2* gene results in lack of sEH expression and reported to produce antiarrhythmic, antihypertensive, and cardioprotective effects.[1,19] On the other hand, sEHi causes inhibition of hydrolase activity exerting similar protective effects in the cardiovascular system.[11,15,20,21] Consistent with the previous observations, the data in this manuscript indicate that both genetic and chemical knockout of sEH lead to a dramatic improvement on the cardiac parameters measure. However, neither approach resulted in full return to normal function indicating that additional approaches could be helpful in restoring cardiac function. The fact that both the chemical and genetic knockout experiments resulted in similar improvements in function indicates that a near maximum effect for a sEHi was reached with *t*-AUCB in this system.

Small molecule inhibitors are valuable tools to study the role of sEH in the pathophysiology of cardiovascular diseases. The first generation of sEHi were epoxide-containing compounds that turned out to be substrates of epoxide hydrolase metabolism.[16] These compounds tended to only have a transient inhibitory effect in vitro and were not effective in vivo.[16,22] Epoxide hydrolase inhibitory properties were

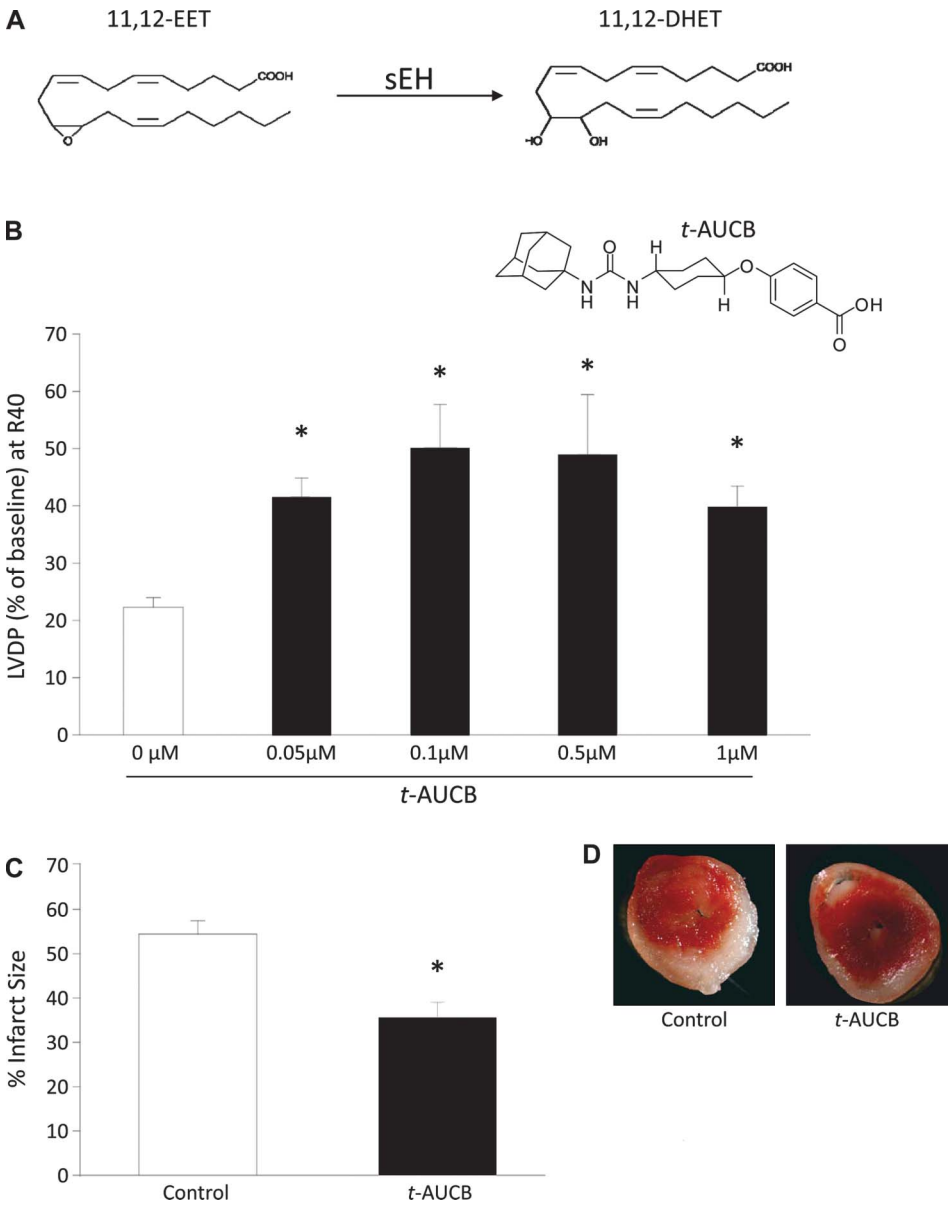

FIGURE 1. Cardioprotective effects of *t*-AUCB. A, Metabolism of 11,12-EET to 11,12-DHET by sEH. B, LVDP recovery at 40 minutes of reperfusion in WT hearts treated with *t*-AUCB (0, 0.05, 0.1, 0.5, or 1 μM). Values represent mean ± standard error of mean; n = 4–11 per group; **P* < 0.05 vs. control. C, Quantification of infarct size. Values represent mean ± standard error of mean; n = 5; **P* < 0.05 versus control. D, Representative images of TTC staining in *t*-AUCB and vehicle control hearts. Surviving tissue stained red with TTC and infracted white tissue.

shown with urea, amide, and carbamate functionalities as the central pharmacophore. The urea was in general the most active with similar R groups, but amide and carbamate derivatives with optimized substituents can be as active as ureas.[16] The next generation of sEHi demonstrated that 1,3-disubstituted urea derivatives were metabolically stable and more potent sEHi than previous compounds.[16,23] *t*-AUCB has a lipophilic adamantyl group on one of nitrogen atoms of the urea and a benzoic acid group linked by a cyclohexyloxy group, which dramatically increase water solubility.[24–26] Formation of hydrogen bonds between urea group and residues at active sites of sEH results in inhibition of hydrolase activity.[16] sEH has a high V_{max} and low K_M for endogenous epoxides of arachidonic and linoleic acid, which results in diminished biological activity. Emerging evidence demonstrates that sEHi are potential therapeutic compounds for the treatment of several diseases. *cis*-isomer of *t*-AUCB (*cis*-4-[4-(3-adamantan-1-y1-ureido)-cyclohexyloxy]-benzoic acid; *c*-AUCB) has recently been demonstrated to attenuate monocrotaline-induced pulmonary hypertension in rats.[27] Xu et al[28] has reported importance of sEH inhibition, which prevented and reversed cardiac hypertrophy using 1-adamantan-3-(5-(2-(2-ethylethoxy)ethoxy) pentyl)urea, AEPU. Similarly, 1-(1-methanesulfonyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)-urea (TUPS) can block angiotensin-II–induced cardiac hypertrophy.[20] 1,3-disubstituted urea sEHi such as AUDA and AUDA-nBE have demonstrated protective effects against ischemia–reperfusion injury in the micromolar range.[11,15] In the present study, *t*-AUCB exerted cardioprotective effects by inhibiting sEH activity at nanomolar concentrations. Apart from urea-based sEHi, nonurea derivatives of potent sEHi have also been reported; however, no study has demonstrated in vivo effectiveness of these compounds.[29,30]

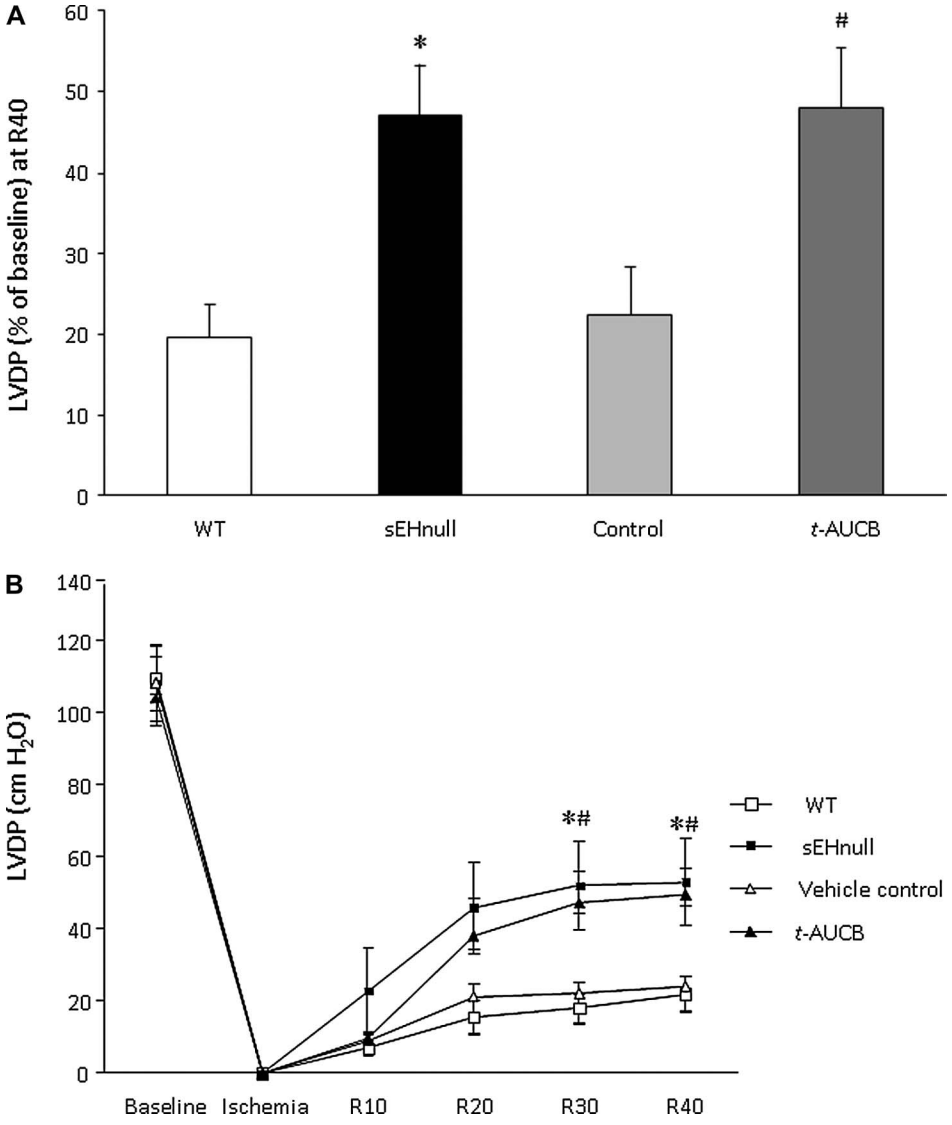

FIGURE 2. Effect of genetic or pharmacological inhibition of sEH on postischemic contractile function. A, LVDP recovery at 40 minutes of reperfusion in WT hearts, sEH null, vehicle control, and WT treated with *t*-AUCB (0.1 μM). Values represent mean ± standard error of mean; n = 5–11 per group; **P* < 0.05 versus WT; #*P* < 0.05 versus vehicle control. B, LVDP in perfused hearts from WT, sEH null, vehicle control, and WT treated with *t*-AUCB (0.1 μM). Values represent mean ± standard error of mean; n = 5–11 per group; **P* < 0.05 versus WT; #*P* < 0.05 versus vehicle control.

TABLE 2. Cardiac Parameters for Pharmacological and Genetic Inhibition of SEH

	WT (n = 5)	sEH Null (n = 9)	Vehicle Control (n = 11)	WT + *t*-AUCB (0.1μM) (n = 5)
Isolated perfused heart				
Preischemic				
LVDP (cmH_2O) (baseline)	114.1 ± 9.0	112.5 ± 10.8	112.7 ± 7.9	108.4 ± 3.6
Rate of contraction, dP/dt_{max} (cmH_2O/msec) (baseline)	3384 ± 332	3416 ± 507	3249 ± 294	3361 ± 198
Rate of relaxation, $-dP/dt_{min}$ (cmH_2O/msec) (baseline)	−2920 ± 257	−2809 ± 448	−2683 ± 188	−2786 ± 148
HR, perfused (beats/min) (baseline)	325 ± 28	297 ± 29	291 ± 19	368 ± 17#
Postischemic				
LVDP (cmH_2O) (R40)	22.8 ± 4.7	55.2 ± 12.4*	25.1 ± 2.9	51.5 ± 7.8#
Rate of contraction, dP/dt_{max} (cmH_2O/msec) (R40)	587 ± 142	1377 ± 162*	807 ± 92	1549 ± 306#
Rate of relaxation, $-dP/dt_{min}$ (cmH_2O/msec) (R40)	−578 ± 119	−1291 ± 181*	−688 ± 82	−1387 ± 194#
HR, perfused (beats/min) (R40)	244 ± 42	251 ± 23	323 ± 16	333 ± 18

Hemodynamic parameters were measured in isolated perfused hearts. Values represent mean ± standard error of mean.
**P* < 0.05 versus WT; #*P* < 0.05 versus vehicle control.
HR, heart rate; R40, 40 minutes of reperfusion.

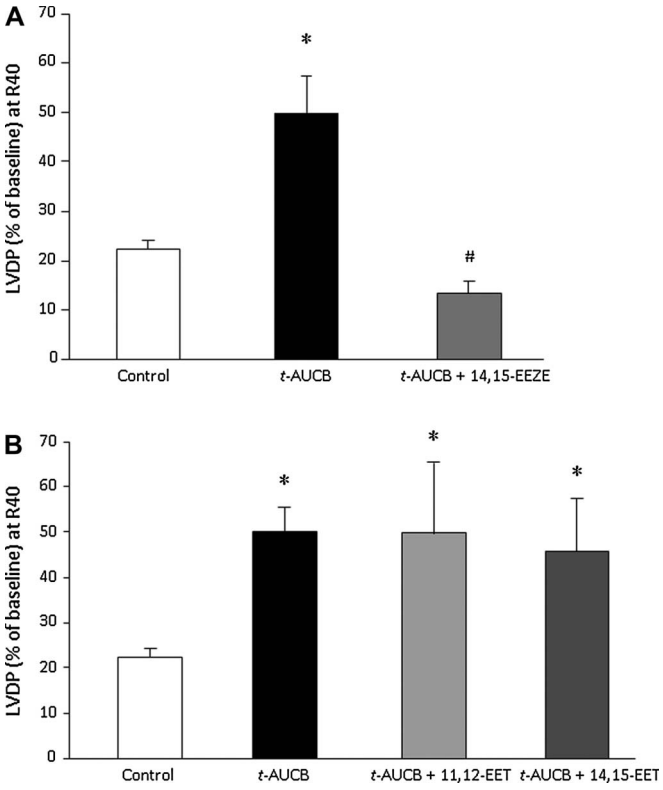

FIGURE 3. EET mediated cardioprotection in *t*-AUCB–treated hearts. A, LVDP recovery at 40 minutes of reperfusion in WT hearts treated with *t*-AUCB (0.1 μM) in presence or absence of putative EET-receptor antagonist, 14,15-EEZE (10 μM). Values represent mean ± standard error of mean; n = 5 per group; *$P < 0.05$ versus vehicle control; #$P < 0.05$ versus *t*-AUCB–treated hearts. B, LVDP recovery at 40 minutes of reperfusion as percentage of baseline. Hearts were perfused with *t*-AUCB (0.1 μM) in presence or absence of exogenous 11,12-EET (1 μM) and 14,15-EET (1 μM). Values represent mean ± standard error of mean; n = 3–5 per group; *$P < 0.05$ versus vehicle control.

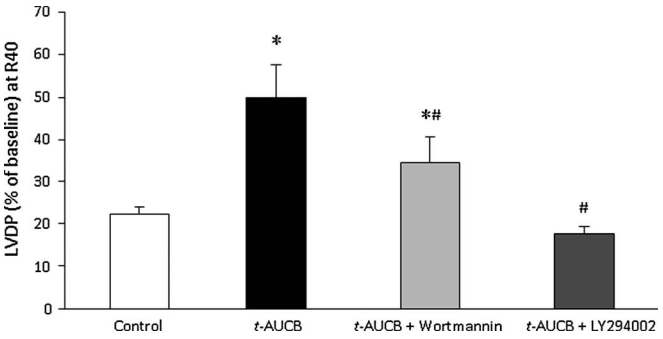

FIGURE 4. Role of PI3K in *t*-AUCB mediated cardioprotection. LVDP recovery at 40 minutes of reperfusion as percentage of baseline. Hearts were perfused with *t*-AUCB (0.1 μM) in presence or absence of PI3K inhibitor wortmannin (200 nM) or LY294002 (5 μM). Values represent mean ± standard error of mean; n = 5 per group; *$P < 0.05$ versus vehicle control; #$P < 0.05$ versus *t*-AUCB–treated hearts.

Cardioprotective effects toward ischemia–reperfusion injury by sEHi compounds have only been demonstrated with AUDA and AUDA-nBE.[11,15] These sEHi compounds have several drawbacks, which include limited oral bioavailability, instability due to rapid metabolism, and poor physical properties such as low water solubility.[18] With very careful formulation, AUDA and its salts and esters can be orally administered for in vivo use, but the new generation of sEHi such as *t*-AUCB are dramatically easier to administer and have longer half-lives.[5,11,15,18,31] *t*-AUCB (IC_{50} = 1.3 nM) is a more potent inhibitor of sEH than AUDA (IC_{50} = 3 nM) and AUDA-nBE (IC_{50} = 7 nM), whereas it is equipotent to *N*-(1(2,2,2-trifluoroethanoyl)piperidin-4-yl)-*N′*-(adamant-1-yl)urea) (IC_{50} = 1.1 nM) and *c*-AUCB (IC_{50} = 0.89 nM).[26] Liu et al[24] compared AUDA-BE to *t*-AUCB for total epoxide and diols in plasma of lipopolysaccharide-treated mice. Both, AUDA-BE and *t*-AUCB, significantly reduced DHET formation increasing the epoxide to diol ratio. However, *t*-AUCB was able to increase the EET:DHET ratio to similar levels of AUDA-BE at a 100 times lower dose. *t*-AUCB has better oral bioavailability and metabolic stability as higher area under concentration–time curve (AUC_t) and longer elimination half-life ($t_{1/2}$) (AUC_t = 155 μM/L; $t_{1/2}$ > 1400 minutes) compared with AUDA (AUC_t = 24 μM/L; $t_{1/2}$ = 575 minutes) and AUDA-BE (AUC_t = 16 μM/L; $t_{1/2}$ = 260 minutes).[18,24,26,31] Similarly, pharmacokinetic studies done in a canine model revealed that *t*-AUCB was found in the circulation for 1 day after oral administration.[18] The improved physical and pharmacokinetic properties of *t*-AUCB make it an attractive therapeutic agent and experimental probe for increasing beneficial EETs.

Inhibition of sEH decreases metabolism of EETs and thereby exerts protective effects against injury incurred after myocardial ischemia and reperfusion and ischemic stroke.[1,11,15,17] Increased EETs in the heart activate various cardioprotective pathways leading to improved contractile function and reduced damage to the heart.[1,11,13] The cardioprotective effects of EETs and sEHi can be blocked by simultaneous treatment with putative EET receptor antagonist, 14,15-EEZE.[1,9–11] Consistent with these observations, our data suggest EETs mediate the protective effects of *t*-AUCB, as the improved postischemic functional recovery was abolished by coperfusion with 14,15-EEZE. It was surprising that addition of EETs to this system did not further increase cardiac function. This may be the result of sufficient amounts of EETs, other omega-6 or 3 epoxides free or esterified in cardiac tissue, or the rate of synthesis of epoxides is high enough that supplementation is not needed. Recent evidence suggests that EETs can activate PI3K/Akt signaling pathway, which leads to inhibition of glycogen synthase kinase-3β and reduces the damage to mitochondria.[1,10] Dhanasekaran et al[31] reported EET-mediated activation of multiple antiapoptotic targets through PI3K/Akt survival signaling. Moreover, inhibition of PI3K pathway by wortmannin or LY294002 abolished the improved postischemic functional recovery of sEH null mice.[1] Similarly, our results show significant reduction in postischemic functional recovery of hearts coperfused with *t*-AUCB and

PI3K inhibitor wortmannin or LY294003, which confirms the involvement of PI3K survival signaling.

CONCLUSIONS

In conclusion, we report improved postischemic contractile function and reduced infarct size by treatment with a sEHi, *t*-AUCB. Moreover, the cardioprotective effect was attributed to EETs and the PI3K survival pathway. The increased potency and water solubility of *t*-AUCB over other sEHi suggest that this compound may serve as a potential therapeutic agent in myocardial ischemia–reperfusion injury and as a potentially a valuable drug for treatment of ischemic heart diseases.

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