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Soluble epoxide hydrolase: A new target for cardioprotection

Garrett J Gross* and **Kasem Nithipatikom**
Medical College of Wisconsin, Department of Pharmacology and Toxicology, 870l Watertown Plank Road, Milwaukee, WI 53226, USA

Abstract

Arachidonic acid is metabolized to a number of bioactive eicosanoid molecules by several enzyme, including enzymes of the COX, lipoxygenase and cytochrome P450 (CYP) monooxygenase pathways. Inhibition of the CYP ω-hydroxylase pathway, stimulation of the CYP-epoxygenase pathway and administration of exogenous epoxyeicosatrienoic acids resulted in cardioprotection in animal models of ischemia; contractile function was improved in mouse hearts subjected to global ischemia/reperfusion, and infarct size was reduced in canine and rat hearts. Cardioprotective effects were also achieved when metabolism of the endogenous epoxyeicosatrienoic acids (EETs) by their major enzymatic hydrolysis pathway was blocked in gene knockout mice ($EPHX2^{-/-}$) or by inhibitors of soluble epoxide hydrolase (sEH), such as 12-(3-odamantan-l-yl-ureido)-dodecanoic acid (AUDA). Pretreatment of canine hearts with AUDA dose-dependently reduced infarct size, and AUDA enhanced the infarct-sparing effect of treatment with exogenous EETs. The preliminary results of studies in rodent hearts have also demonstrated that AUDA and AUDA-butyl ester reduce infarct size. These results and others obtained in models of myocardial stunning and hypertrophy suggest that inhibitors of EPHX2 or sEH have therapeutic potential in a brood range of cardiovascular diseases.

Keywords

Coronary heart disease; cytochrome P450; heart disease; ischemia; pharmacology

Introduction

Cardiovascular disease (CVD) is the leading cause of mortality in the developed world. In 2007, the overall cost of CVD in the US alone was approximately $432 billion [1]. One of the most common types of CVD is myocardial infarction (MI), occurring in approximately 800,000 individuals annually [1]. Therefore, it is imperative that new therapies are developed to slow the onset of coronary artery disease, to prevent MI, and to ameliorate the severity of damage to the heart by limiting infarct size after the onset of the attack. In 1986, the discovery of the phenomenon of ischemic preconditioning (IPC) by Murry *et al* stimulated great hope for the development of novel therapies [2]. It was demonstrated that brief periods of ischemia prior to a more prolonged bout of ischemia could markedly reduce infarct size in dogs and, subsequently, in all animals tested and in humans [2]. Although a number of drugs mimic IPC in animal models, however, no treatment has emerged that is effective in all patients experiencing an acute MI. The primary reason that IPC or pharmacological drugs to mimic IPC are inappropriate as standard treatments for patients suffering MI is that IPC is only effective if administered prior to the ischemic insult, which is almost impossible to predict.

*To whom correspondence should be addressed.

A resurgence of excitement has recently occurred in the field of cardiovascular medicine with of the discovery of the phenomenon of postconditioning, Vinten-Johansen *et al* demonstrated in dogs that if reperfusion following a prolonged ischemic period is conducted in a 'stuttering' fashion, with alternate cycles of 3- to 30-sec reperfusion and occlusion, infarct size is reduced by a magnitude similar to that observed with IPC [3]. Importantly, the advantage of this technique, or pharmacological means to mimic postconditioning, is that, unlike IPC, the treatment can be administered at the time of reperfusion. These findings caused a paradigm shift in the field of ischemia/reperfusion and fostered efforts to develop a safe drug that can reduce myocardial injury when administered just prior to or at the time of reperfusion. This review discusses the potential use of selective soluble epoxide hydrolase (sEH) inhibitors, such as 12-(3-adamantan-l-yl-ureido) dodecanoic acid (AUDA), as a potential new therapeutic approach in the treatment of reperfusion injury.

Soluble epoxide hydrolase

The cytochrome P-450 (CYP) monooxygenase pathway metabolizes arachidonic acid to produce two types of eicosanoid molecules, hydroxyeicosatetranoic acids (HETEs) resulting from the action of CYP hydroxylases and epoxyeicosatrienoic acids (EETs) resulting from the action of CYP-epoxygenases [4], Four regioisomers of EETs are known - 5,6-EET, 8,9-EET, 11,12-EET and 14,15-EET - and these share many biological effects, with the exception of 5,6-EET. EETs and HETEs often exert opposing effects, particularly in the tissues of the heart in which EETs are vasodilators and have several cardioprotective effects [5,6], whereas HETEs (in particularly 20-HETE) produce coronary artery vasoconstriction and increase infarct size in experimental models [7]. An important feature of EETs is that these molecules are metabolized by a specific enzyme, sEH, to the corresponding dihydroxyeicosatrienoic acids (DHETs). DHETs are generally much less efficacious at causing vasodilation than their corresponding precursory EETs in most systems and models studied, although DHETs may exert important effects in some organs [4]. In this regard, Morisseau *et al* synthesized several urea and carbamate compounds as potent sEH inhibitors, one of which was AUDA [8]. These inhibitors enhanced the cytotoxicity of trans-stilbene oxide and reduced the toxicity of leukotoxin *in vitro*, reduced the toxicity of leukotoxin *in vivo* in mice, and prevented the symptoms of acute respiratory distress syndrome. These data suggested that these compounds may have efficacy in treating various inflammatory conditions in which epoxides and diols may be involved. Additional interest in developing selective sEH inhibitors arose as a result of studies in which the genetic knockout of *EPHX2* (the gene encoding sEH) in mice caused a decrease in baseline blood pressure compared with corresponding wild-type mice [9]. These findings suggested that selective sEH inhibitors might be useful as treatments for hypertension, and possibly other cardiovascular disorders [9].

New data suggest that selective sEH inhibitors, such as AUDA, are cardioprotective in several models of ischemia/reperfusion injury [10–13]. Compared with wild-type animals, mice in which CYPZJ2 is overexpressed or sEH is inactivated have a superior recovery of contractile function in reversibly injured hearts and in infarct size after ischemia, and a decrease in the incidence of cardiac arrhythmias [10,13]. In dogs, treatment with AUDA reduced infarct size in a dose-dependent manner and enhanced the cardioprotective effects of exogenously administered EETs [11]. Similar results have been observed using the selective sEH inhibitor AUDA-butyl ester (AUDA-BE) in C57BL/6J wild-type mice [12].

The cardioprotective effects of AUDA and sEH expression (sEH knockouts)

Effects of sEH expression on reversible myocardial contractile dysfunction in mice

Seubert *et al* were the first research group to study the role of sEH on the recovery of contractile function in Langendorff-perfused hearts [10]. In this study, mice with a knockout of the sEH

gene had an improved recovery of left ventricular-developed pressure (LVDP) following 20 min of global ischemia and 40 min of reperfusion [10]. In a subset of these knockout mice, a modest but statistically significant reduction in infarct size was demonstrated by tetrazolium staining and by the measurement of lactate dehydrogenase (LDH) release. These beneficial effects were accompanied by an increase in epoxide:diol ratios in the plasma of the knockout mice, which suggested an increase in EETs were present in the sEH knockouts. In further studies, the selective EET antagonist 14,15-epoxyeicosa-5(Z)-enoic acid (14,15-EEZE) blocked the beneficial effects observed in the sEH knockout mice [14]. The perfusion of isolated wild-type murine hearts with physiologically relevant concentrations of 8,9-EET, 11,12-EET and 14,15-EEf resulted in an improvement in the recovery of LVDP that was similar to that observed in the knockout mice [14]. These findings suggest that the beneficial effects observed on contractile function and infarct size were the result of increased EET concentrations in this Langendorff model.

The beneficial effects on contractile function observed in sEH null mice were also blocked by the phosphoinositol-3-kinase (PI3K) inhibitors wortmannin and LY-294002; the K_{ATP} channel blockers glibenclamide and 5-hydroxydecanoic acid; and the calcium-activated potassium channel inhibitor paxilline [10]. All of the intracellular events were accompanied by increases in phospho-glycogen synthase kinase-3β (p-GSK3β), which has demonstrated cardioprotective effects in several other studies [15,16]. GSK3β protects the heart from injury by delaying or blocking the opening of the mitochondrial permeability transition pore [16].All of these potential pathways by which the EETs and sEH inhibitors produce cardioprotection are summarized in Figure 1. Taken together the results of these studies suggest that a pharmacological inhibitor of sEH, such as AUDA, should exert cardioprotective activity similar to that observed in sEH null mice.

Effects of AUDA on myocardial infarct size AUDA in dogs

Exogenous EETs produce a marked reduction in infarct size in dogs [17]; however the enhancement of endogenous EET concentration by inhibiting the breakdown of EETs by sEH has not been demonstrated to produce a similar response. Because the cardiovascular effects of EETs on the heart are presumed to be the result of actions on a putative EET receptor on the cell membrane or within the cell, studies were conducted to assess whether selective EET antagonists, such as 14,15-EEZE, could block the effect of several exogenous EETS or the endogenous effect of AUDA [8]. As illustrated in Figure 2, intracoronary administration of AUDA (0.157 or 0.314 mg/kg) produced a dose-related reduction in infarct size (IS), expressed as a percentage of the area at risk (AAR) [11]. The higher dose of AUDA produced a reduction in infarct size that was approximately equivalent to that produced by intracoronary-administered exogenous 14,15-EET [11]. Interestingly, when the lower dose of AUDA was co-administered with 14,15-EET, the reduction in IS/AAR was significantly greater than that observed when the small dose of AUDA was administered alone, and was larger than that observed when 14,15-EET was administered alone (Figure 3) [11]. These data suggest that these effects of AUDA were the result of enhanced EET concentrations in the heart. AUDA caused a dose-related increase in the release of 14,15-EET into the coronary venous blood, draining the previously ischemic area at 5 and 30 min of reperfusion. The selective EET antagonist 14,15-EEZE blocked the effects of both the exogenously administered 14,15-EET and AUDA; the inhibition of AUDA increased endogenous EET concentrations [11]. In contrast, the cardioprotective effects of the mitochondrial K_{ATP} opener diazoxide were not inhibited by 14,15-EEZE. These data suggest that 14,15-EEZE is selective in inhibiting the effects of exogenous and endogenous EETs; however, the results obtained thus far do not determine the site of EET action, whether extracellular or intracellular. Perhaps the EETs have several sites of action that result in the overall cardioprotective effect observed. Further

experiments are needed to ascertain whether single or multiple receptors are involved in mediating EET-induced cardioprotection.

AUDA-BE in mice

In studies in male C57BL/6J mice subjected to 40 min of left coronary artery occlusion and 2 h of reperfusion, the IS/RRR was 46 ± 3% [12]. However, in similar wild-type mice pretreated with AUDA-BE (ip) 30 min prior to occlusion, IS/AAR was reduced to 30 ± 5% ($p < 0.01$) [12]. Although EETs were not measured in these studies, the researchers presume that the cardioprotective effect of AUDA-BE was the result of increases in endogenous EET concentration in the drug-treated mice [12]. This seems likely because the protective effect of AUDA-BE was completely blocked by the EET antagonist 14,15-EEZE, and was mimicked by the intravenous administration of 14,15-EET, a major substrate of sEH [12].

Effect of soluble epoxide hydrolase inhibitors in the prevention and reversal of cardiac hypertrophy: A possible role in heart failure

The most common consequence of an acute MI is the development of ischemic cardiomyopathy and hypertension that results in ventricular remodeling and hypertrophy. Eventually these changes result in severe heart failure and often in sudden death from ventricular fibrillation. To address the role of sEH in the development of left ventricular hypertrophy (LVH), Xu *et al* studied several groups of C57BL/6J mice with pressure overload-induced LVH [18]. Mice were administered two potent sEH inhibitors, 1-adamantan-1-yl-3-(5-(2-(2-ethoxyethoxy) ethoxy)pentyl)urea (AEPU) and AUDA, either before the onset of LVH or following the establishment of LVH, In both groups, the sEH inhibitors were cardioprotective and prevented the development of LVH or reversed pre-established LVH. Interestingly, both compounds prevented the activation of NFKB, a mediator of LVH development in cardiac myocytes. Furthermore, these two sEH inhibitors exerted an antiarrhythmic effect, in association with their beneficial effects of preventing LVH [18]. These investigators concluded that the use of sEH inhibitors may increase levels of endogenous lipids, such as the EETs, and may have therapeutic potential in the prevention of LVH and heart failure following activation of NFKB and its subsequent remodeling pathway.

More recently, Monti *et al* established that the allelic variation of *EPHX2* is associated with heart failure in a rat model of heart failure [19]. Increased expression of *EPHX2* transcript and protein, and higher enzyme activity, resulted in the more rapid breakdown of cardioprotective EETs. In *EPHX2* gene knockout mice, the mice hearts were protected from developing pressure overload-induced heart failure and cardiac arrhythmias. Taken together, these results suggest that inhibition of sEH may be a new therapeutic approach to add to the increasing pharmacotherapy for heart failure [20]. A more detailed description of the role that sEH inhibitors play in the development of heart failure and the role of NFKB is included in two reviews by Imig [21] and Harris *et al* [22].

Soluble epoxide hydrolase gene deletion reduces survival after cardiac arrest and cardiopulmonary resuscitation

Hutchens *et al* recently tested the hypothesis that sEH knockout mice would be less susceptible to transient whole-body cardiac arrest (10 min) and resuscitation [23]. Surprisingly, survival in the wild-type mice was significantly greater than in the sEH knockout mice. Equivalent studies with selective sEH inhibitors have not been conducted, but might provide a different result because the sEH enzyme is a bifunctional enzyme in which the C-terminal domain contains the hydrolase activity and the N-terminal domain contains a functional phosphatase [24], which is not blocked by the sEH inhibitors. Therefore, it is possible that a sEH inhibitor that only blocks the C-terminal domain might have a beneficial effect in this setting.

Conclusion

Although understanding of the potential of EETs and sEH inhibitors as cardioprotective agents in a number of CVDs is in its infancy, as noted by the modest number of publications at this time, the results that are emerging are encouraging. Findings suggest that methods for increasing levels of endogenous EETs by inhibiting their breakdown through the use of sEH inhibitors produce a number of beneficial effects on the reversibly and/or irreversibly injured heart and in other organs [21,22]. The results obtained thus far suggest that the enhancement of endogenous EET concentrations or inhibition of the breakdown of exogenously administered EETs (by sEH inhibitors or by the administration of novel EET agonists) can have several benefits. These include a reduction in myocardial stunning, myocardial infarct size and inflammatory response, prevention of the onset of LVH and subsequent remodeling, which leads to heart failure, and reductions in the incidence of cardiac arrhythmias associated with heart failure. There are few drugs, such as the PDE5 inhibitors (eg, sildenafil), that seem to possess these wide-ranging beneficial effects on the heart and circulation in the absence of any noticeable side effects. However, this group of compounds should be tested in conscious animal models and models of disease and aging because many compounds that are effective in young healthy animals are less effective in older animals or those with diseases such as diabetes and the metabolic syndrome [25]. Thus far, the beneficial effects of sEH inhibitors occurred in the absence of any significant side effects; however well-controlled clinical trials are needed to further assess the safety of this group of compounds before they can be considered for clinical use. It might be more likely that these compounds are most useful in acute situations, such as coronary angioplasty or bypass surgery, and not in situations in which treatment would be required for a prolonged period of time. The observation that these compounds are effective when administered just prior to reperfusion is a significant advantage because there is the potential for use in patients presenting with an ongoing infarction, where drug treatment can be administered at the time of reperfusion. Further clinical studies are needed to assess the potential use of sEH inhibitors, such as AUDA, in these various cardiovascular disorders.

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•• of outstanding interest

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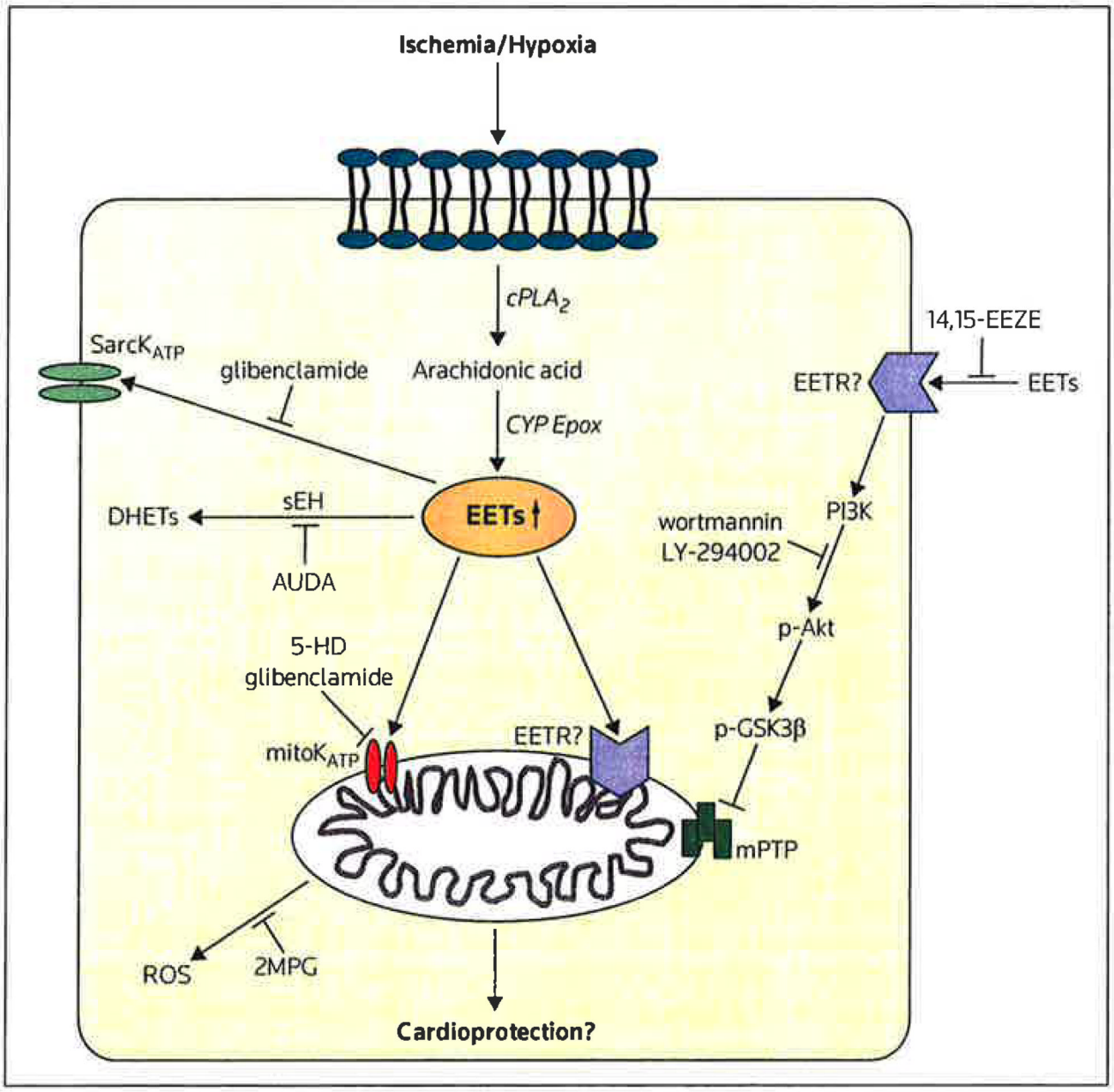

Figure 1. Mechanistic pathways of soluble epoxide hydrolase inhibitors and/or epoxyeicosatrienoic acids in cardioprotection

Several potential pathways for the activity of soluble epoxide hydrolase (**sEH**) inhibitors have been identified that may be involved in epoxyeicosatrienoic acid (**EET**)-induced cardioprotection. Because there is evidence that the EETs may exert both extracellular and intracellular effects, it is postulated that there may be an EET receptor (**EETR**) at the cell surface or within the mitochondria; however, no EET receptor has been cloned and this hypothesis remains questionable.

2MPG 2-mercaptopropionyl glycine, **5-HD** 5-hydroxydecanoic acid, **14,15-EEZE** 14,15-epoxyeicosa-5(Z)-enoic acid, **AUDA** .12-(3-adamantan-1-yt-ureido) dodecanoic acid, **cPLA$_2$** cytosolic phosphotipase A$_2$, **CYP Epox** cytochrome P-450 epoxygenase, **DHETs** dihydroxyeicosatrienoic acids, **mitoK$_{ATP}$** mitochondrial ATP-sensitive potassium channel,

mPTP mitochondrial permeabitity transitionpore, **p-Akt** phosphorylated Akt, **p-GSK3**β phospho-gtycogen synthase kinase-3β, **Pl3K** phosphoinositol-3-kinase, **ROS** reactive oxygen species, **SarcK$_{ATP}$** sarcolemmal ATP-sensitive potassium channel

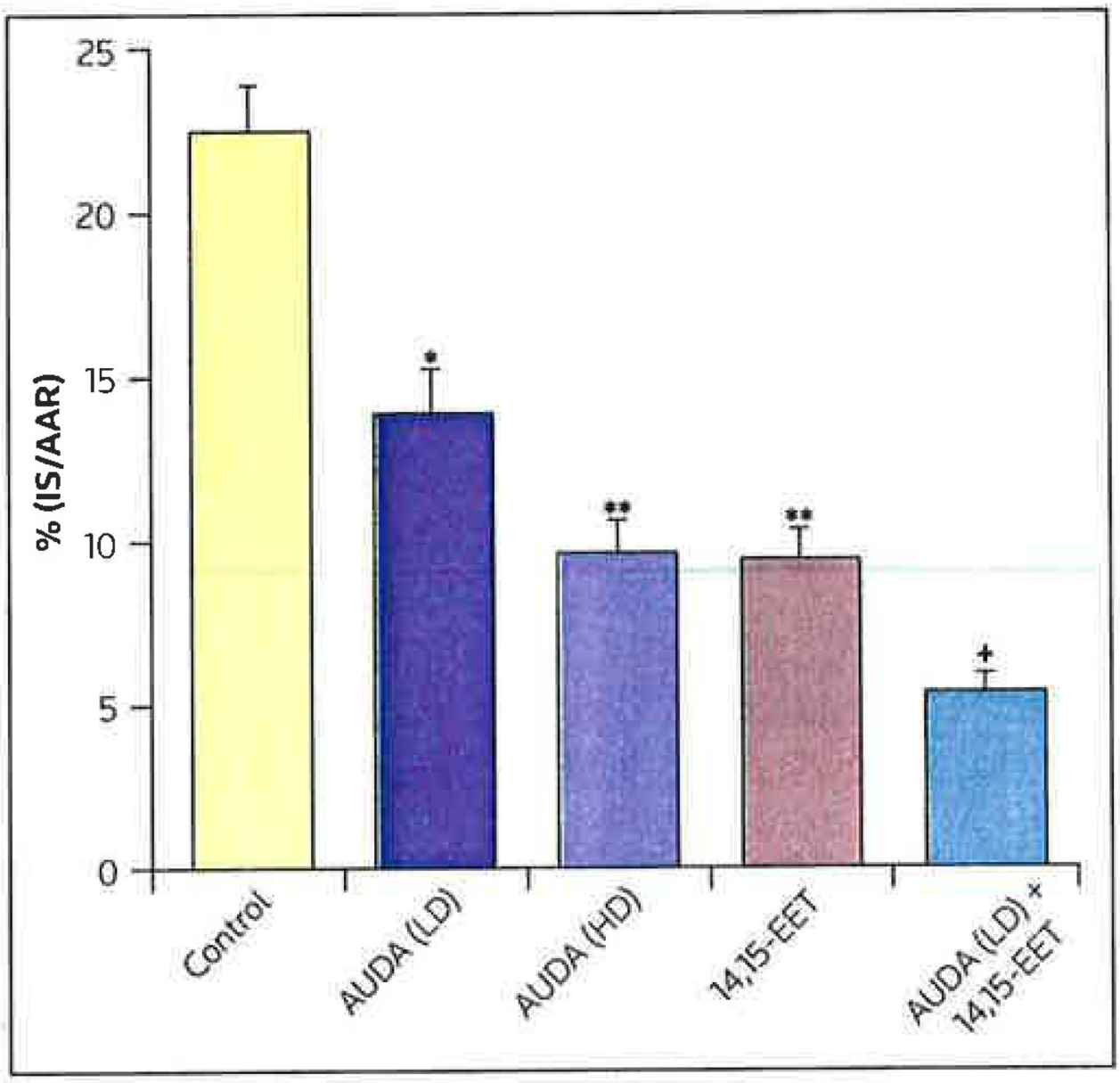

Figure 2. The effects of AUDA and 14,15-EET on myocardial infarct size

Effects of Low-dose (**LD**; 0.157 mg/kg) and high-dose (**HD**; 0.3,l4 mg/kg) l2-(3-adamantan-l-yl-ureido) dodecanoic acid (**AUDA**), and 14,15-epoxyeicosatrienoic acid (**14,15-EET** 0.128 mg/kg) atone or in combination with LD AUDA on myocardial infarct size as a percentage of the area of risk (**IS/AAR**) in dogs subjected to 60 min of left anterior descending coronary artery occlusion and 3 h of reperfusion.

+p < O.O5 compared with LD AUDA, *p < 0.01 compared with control, **p < 0.00.l compared with control

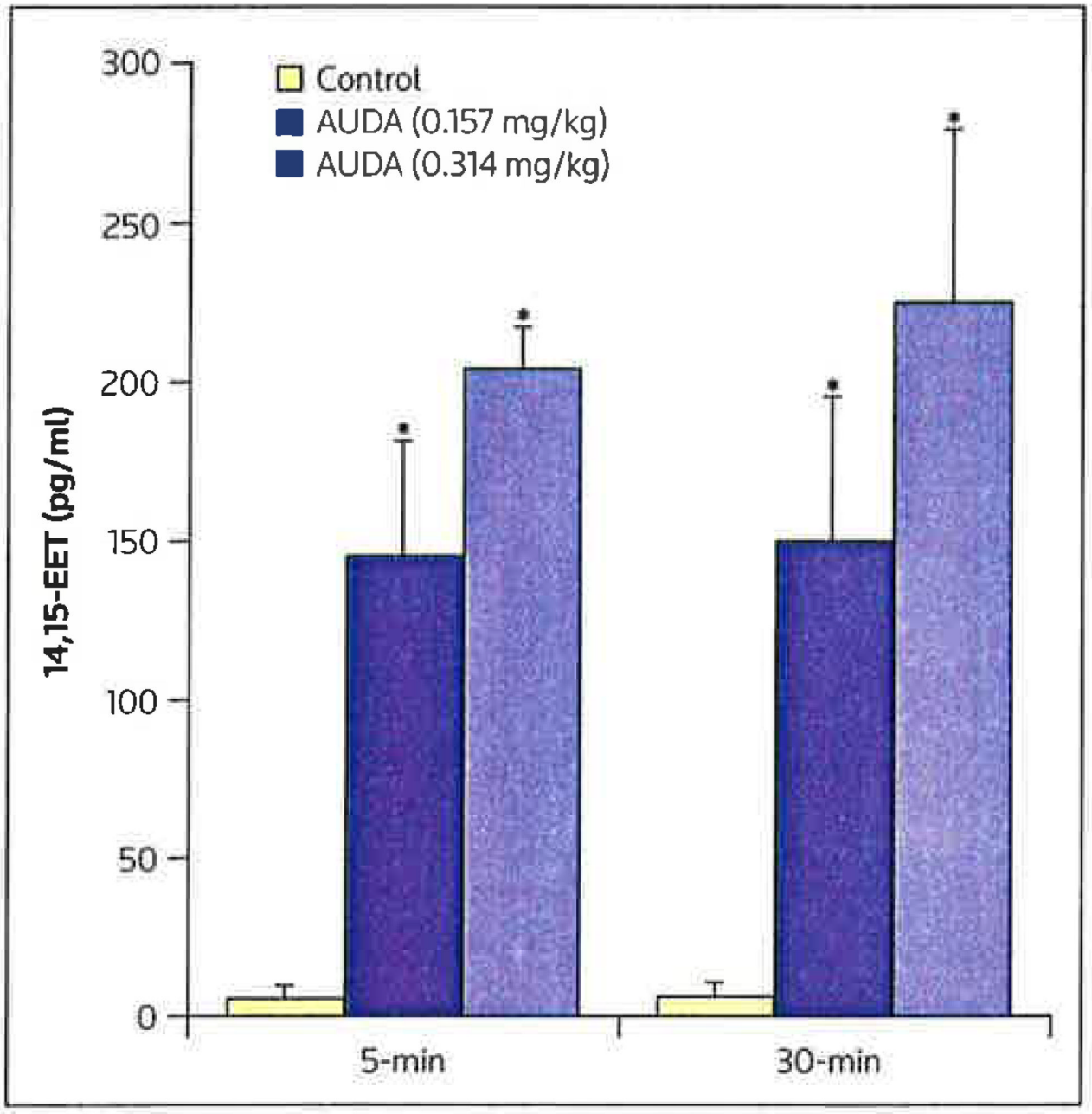

Figure 3. Changes in 14,15-EET concentrations in the plasma with AUDA treatment

Concentrations of 14,15-epoxyeicosatrienoic acid (**14,15-EET**) in the coronary venous plasma at 5 and 30 min of reperfusion following treatment with low or high doses of 12-(3-adamantan-1-yl-ureido) dodecanoic acid (**AUDA**; 0.157 and 0.314 mg/kg, respectively) compared with control values.

*$p < 0.01$ compared with controls