Special Report

Not so EEZE: The 'EDHF' antagonist 14, 15 Epoxyeicosa-5(z)-enoic Acid has vasodilator properties in mesenteric arteries

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1. Summary

P-450 metabolites, including the epoxyeicosatrienoic acids, are likely candidates for endothelial derived hyperpolarising factor (EDHF). In the present study, we confirm that the stable analogue 11-nonyloxyundec-8(Z)-enoic acid is a vasodilator of murine vessels. However, we also show that the 'epoxyeicosatrienoic acid receptor' antagonist 14, 15 EEZE similarly dilates murine vessels contracted with U46619, prostaglandin $F_{2\alpha}$ or methoxamine, but not with endothelin-1 or potassium. We suggest that 14,15 EEZE is a partial agonist for the epoxyeicosatrienoic acids/EDHF receptor. These results illustrate an important pharmacological property of this antagonists, which is being increasingly used to study the nature of EDHF.

Key words: EDHF; 14, 15 EEZE; hyperpolarisation; U46619; mouse mesenteric artery.

2. Introduction

The endothelium plays a key role in the control of vasomotor tone via the release of diffusible factors such as prostanoids, nitric oxide, and endothelial derived hyperpolarising factor (EDHF). The identity of EDHF is controversial, although there has been considerable support for a role of cytochrome P450 (CYP) metabolites (Archer et al., 2003; Campbell et al., 1996; Fisslthaler et al., 1999). However, the specific pharmacological tools required for investigating this theory have only recently been made available (Falck et al., 2003; Gauthier et al., 2002).

Epoxyeicosatrienoic acids are CYP metabolites, which induce vasodilatation by activating smooth muscle large conductance Ca^{2+} sensitive K^+ (BK_{Ca}) (Archer et al, 2003). The epoxyeicosatrienoic acids are currently thought to be important mediators in EDHF responses. Specifically, acetylcholine induced vasodilatation, in human and bovine tissue, is inhibited by P450 inhibitors and by the epoxyeicosatrienoic acid analogue 14, 15-epoxyeicosa-5 (Z) enoic acid (14, 15 EEZE) (Archer et al, 2003; Gauthier et al, 2002). 14, 15 EEZE may therefore represent the first EDHF antagonist and consequently is a very useful pharmacological tool in the study of vascular biology. However, a full pharmacological analysis of 14, 15, EEZE has not been made. In the present study we present data which shows that 14, 15 EEZE is a vasodilator in its own right. In fact, 14, 15 EEZE was more potent a vasodilator than the 'agonist' 11-nonyloxyundec-8(Z)-enoic acid (Falck et al., 2003).

3. Methods

Male Black 6 C57 mice (28.2 ± 1.24g) were killed by lethal exposure to CO_2. The mice were maintained and killed in accordance with the European Community guidelines for the use of experimental animals. The mesenteric bed was removed using ligatures, and placed into physiological salt solution (PSS; composition in mM) NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, EDTA 0.027, and Glucose 5.5. Segments of first order mesenteric artery were removed and mounted in a four channel Mulvany-Halpern myograph under normalised tension (Mulvany and Halpern, 1977). In this study, the second order arteries had a mean internal diameter of 226μm. The vessels were equilibrated to 37^{o}C and the solution gassed with 95% O_2 and 5% CO_2 for 30 minutes. The arterial segments were challenged twice with high potassium solution (KPSS; composition in mM: KCl 123.7, $CaCl_2$ 2.5, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, EDTA 0.027, and Glucose 5.5), after washing either 9, 11-Dideoxy-11α, 11α-epoxymethanoprostaglandin $F_{2\alpha}$ (U46619), prostaglandin $F_{2\alpha}$, methoxamine or endothelin 1 was added in a cumulative fashion in order to determine the EC_{80} concentration of each drug to induce contraction. The presence of an intact endothelium was confirmed by the ability of 10^{-5}M acetylcholine to induce >70% relaxation of U46619 (EC_{80}) precontracted vessels. Occasionally vessels failed this test and were disregarded.

3.1 *Effects of the agonists 11-nonyloxyundec-8(Z)-enoic acid or the antagonists 14, 15 EEZE on vasodilator effects in pre-contracted vessels*

Vessels were precontracted with the EC_{80} concentration of U46619 followed by the cumulative addition of either 11-nonyloxyundec-8(Z)-enoic acid or 14, 15 EEZE. In some experiments, vessels were pre-contracted with 10^{-5}M methoxamine. The

inhibitory effects of 14, 15 EEZE were investigated by incubation in the presence of 10^{-5}M 14, 15 EEZE for 30 minutes prior to the contraction with U46619 (10^{-8}M – 10^{-6}M) or methoxamine ($3x10^{-8}$M – 10^{-4}M). In experiments where 10^{-5}M 14, 15 EEZE was used, 11-nonyloxyundec-8(Z)-enoic acid ($3x10^{-9}$M – $3x10^{-5}$M) was added in a cumulative fashion after contraction with U46619.

3.2 Materials

All drugs were purchased from Sigma Chemical Co. Gillingham, Dorset, UK, except for 11-nonyloxyundec-8(Z)-enoic acid and 14, 15-EEZE, which were a gift generously provided by J. R. Falck (Department of Pharmacology and Toxicology, University of Texas South-western Medical school, Dallas).

Acetylcholine and methoxamine solutions were freshly prepared each day in aqueous solutions. 14, 15-EEZE, 11-nonyloxyundec-8(Z)-enoic acid, U46619 or $PGF_{2\alpha}$ were prepared in high concentration 'stock' solutions dissolved in ethanol and were stored at -80^{o}C until used. Endothelin-1, dissolved in 0.1% acetic acid was also stored at -80^{o}C in aliquots until used. Where appropriate vehicle (ethanol) was added to control arteries and responses defined as 'time control'.

3.3 Data and statistical analysis

Contractile responses were represented as active effective pressure (AEP; kPa; mN/mm^2) as calculated by the following equations:

$\Delta T = \Delta F/2$ x segment length

AEP = ΔT/(vessel radius)

Where ΔT represents active wall tension and ΔF represents active force response measured in mN.

Relaxant responses were calculated as a percentage of induced tone. Data are given as the mean ± S.E.M. for experiments (1 animal per experiment).

4. Results

4.1 Effects of 14, 15 EEZE on vasodilator responses

U46619 induced concentration dependent vasoconstriction of vessels. Pre-treatment of vessels with 14, 15 EEZE resulted in concentration dependent inhibition of U46619 induced contractions (figure 1a). Similarly, methoxamine induced concentration dependent vasoconstriction of vessels which were also inhibited by 14, 15 EEZE (figure 1b). 14, 15 EEZE appeared more potent an inhibitor of U46619 than methoxamine induced vasoconstriction.

14, 15 EEZE was also able to induce direct and immediate vasodilator responses in vessels contracted with EC_{80} concentrations of U46619 ($3x\ 10^{-8}M$), $PGF_{2\alpha}$ ($10^{-5}M$), or methoxamine ($10^{-5}M$). By contrast, 14, 15 EEZE did not dilate vessels contracted with an EC_{80} concentration of endothelin-1 ($10^{-8}M$) or with depolarising concentrations of potassium ($1.24x10^{-2}M$), (figure 2a).

4.2 Effect of 11-nonyloxyundec-8(Z)-enoic acid on vasodilator responses

11-nonyloxyundec-8(Z)-enoic acid induced vasodilator responses in U46619 contracted vessels with an approximate EC_{50} of approximately $10^{-5}M$ (figure 2b). The dilator effects 11-nonyloxyundec-8(Z)-enoic acid were antagonised by $10^{-5}M$ 14, 15 EEZE (Figure 2b).

5. Discussion

11-nonyloxyundec-8(Z)-enoic acid and 14, 15 EEZE are pharmacological tools used to investigate EDHF candidates. In the present study we show that 11-nonyloxyundec-8(Z)-enoic acid is an effective vasodilator of murine mesenteric vessels. We also show that 14, 15 EEZE is an effective antagonist of these responses. However, we also show that 14, 15 EEZE is a vasodilator with a similar potency to 11-nonyloxyundec-8(Z)-enoic acid .

Vasodilator epoxyeicosatrienoic acids are metabolic products of arachidonic acid breakdown by cytochrome P-450 epoxygenases in the endothelium. However, cytochrome P-450 ω-hydroxylase metabolises arachidonic acid in the smooth muscle cells to form 20-hydroxyeicosatetraenoic acid (20-HETE), which may induce vasoconstriction. Thus, the ratio of arachidonic acid metabolites present in the vessel wall dictates the level of tone of the smooth muscle cells, and any antagonist used to study these enzymes needs to be highly specific. Consequently, Gauthier and co-workers (2002) designed and generated a range of analogues, of which 14, 15 EEZE was found to have important effects. 14, 15 EEZE was found to be a highly specific epoxyeicosatrienoic acid antagonist, with no effect on the action or synthesis of 20-HETE (Gauthier et al., 2000).

It has been proposed that the endothelium synthesises epoxyeicosatrienoic acids which are released upon stimulation by agonist such as acetylcholine. The released epoxyeicosatrienoic acids open large K_{Ca} channels in smooth muscle cells, and thus induce hyperpolarisation and relaxation of the artery (Campbell et al, 1996). However, the vascular type may be sensitive to the type of epoxyeicosatrienoic acid released, since rat renal arteries relax in the presence of 11-nonyloxyundec-8(Z)-enoic acid but 14, 15 epoxyeicosatrienoic acid had little effect (Zou et al., 1996).

The dilator actions of the stable epoxyeicosatrienoic acid analogue 11-nonyloxyundec-8(Z)-enoic acid were abolished by 14, 15 EEZE. This observation is consistent with the notion that 14, 15 EEZE is an antagonist of 11-nonyloxyundec-8(Z)-enoic acid and is in line with findings of Archer et al (2003) and Gauthier et al (2002). However, in the current study we have characterised a vasodilator property of 14, 15 EEZE. Specifically we show that 14, 15 EEZE inhibits contractions induced by U46619 or methoxamine. Furthermore, we show that 14, 15 EEZE induces vasodilatation of vessels contracted with U46619 or $PGF_{2\alpha}$. Similar observations have previously been reported using bovine coronary artery as a bioassay (Gauthier et al 2002), although in this tissue dilator effects were very weak. In the current study we also demonstrate that 14, 15 EEZE is an effective, but less potent and less efficacious dilator of vessels contracted with methoxamine. By contrast, 14, 15 EEZE did not dilate vessels contracted with either endothelin-1 or high potassium; both of which will depolarise vessels as they contract (Van Renterghem et al., 1989). Under these conditions, hyperpolarising vasodilators are inactive. This data suggests that the vasodilator properties of 14 15 EEZE are mediated, like those of 11-nonyloxyundec-8(Z)-enoic acid, via smooth muscle cell hyperpolarisation.

This data supports the use of 14, 15 EEZE as an epoxyeicosatrienoic acids receptor antagonist. However, our data also illustrates other important pharmacological properties of this drug. Specifically, our data suggest that 14, 15 EEZE is an agonist or partial agonist for the putative epoxyeicosatrienoic acids receptor.

6. Reference List

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7. Figure legends

Figure 1. Effects of pre-incubation with 14, 15 EEZE on contraction with either U46619 (a) or methoxamine (b). Data are given as active effective pressure (AEP; kPa), all values are means ± S.E.M. of 3 to 5 experiments.

Figure 2. Dilator properties of 14, 15 EEZE on vessels contracted with potassium ($1.24x10^{-2}$M; KPSS), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; 10^{-5}M), U46619 ($3x\ 10^{-8}$M), endothelin 1 (10^{-8}M) or methoxamine (10^{-5}M),(a). Antagonistic effects of 14, 15 EEZE (10^{-5}M) on 11-nonyloxyundec-8(Z)-enoic acid induced vasodilation in vessels contracted with U46619 (10^{-7}M), (b). Data are given as percentage of U46619 induced tone; all values are means ± S.E.M. of 3 to 5 experiments.