COMMENT

To b’EET or not to b’EET? That is the question!

ABSTRACT

Since the discovery of endothelium-derived hyperpolarizing factor (EDHF), several different candidates and pathways have been proposed as mediators of endothelium-dependent hyperpolarization of vascular smooth muscle. In particular, there has been considerable support for a role for the cytochrome P450 metabolites, the epoxyeicosatrienoic acids (EETs). However, more recently, this hypothesis has come under severe scrutiny. In this issue of Clinical Science, Passauer et al. demonstrate that an EET cannot be EDHF in the human forearm, and add further to the growing belief that an EET is not a candidate for a ubiquitous EDHF.

The vascular endothelium plays an essential role in the regulation of vascular tone through the release of diffusible vasodilator substances such as nitric oxide (NO), prostacyclin (PGI2) and endothelium-derived hyperpolarizing factor (EDHF). Although the importance of NO and PGI2 in regulating vascular smooth muscle (VSM) tone is well established (for review, see [1]), the identity and physiological significance of EDHF remains controversial.

EDHF is described as the NO- and PGI2-independent component of endothelium-dependent vasodilation that is due to hyperpolarization of VSM [2]. This factor contributes to endothelium-dependent dilation in many mammalian vessels, particularly in the resistance vasculature. Indeed, it is clear that, as the size of the artery diminishes, the contribution that EDHF plays in endothelium-dependent dilation increases [3]. This observation itself is highly suggestive of an important role for EDHF in regulating vascular tone and local blood flow. In addition, a growing body of evidence supports a compensatory role for EDHF, whereby the magnitude of EDHF-mediated endothelium-dependent vasodilation is up-regulated or maintained in inflammatory vascular diseases expressing endothelium dysfunction. In particular, this might occur in an attempt to compensate for deficiencies in endothelial NO synthase (eNOS)-derived NO; a defining characteristic of cardiovascular disorders with associated endothelium dysfunction [4]. Indeed, there are several examples in both animal and human studies where an up-regulated/sustained EDHF response is exposed when vascular eNOS-derived NO activity has been compromised, i.e. eNOS-knockout animals [5], hypercholesterolaemia [6,7], hypertension [8] and congestive heart failure [9]. Therefore the identification of EDHF is likely to be of physiological and pathophysiological significance and may provide a rationale for the design of novel therapeutics for such cardiovascular disorders.

Since the discovery of EDHF [2], several different candidates and pathways have been proposed as mediators of endothelium-dependent hyperpolarization of VSM, including cytochrome P450 (CYP) metabolites, particularly 11,12 epoxyeicosatrienoic acid (EET), K+ ions, H2O2, cyclic AMP, c-type natriuretic peptide (CNP) and also gap junctions. A plethora of reports stand in support or refute each of the potential candidate mechanisms. Much of the differences in the literature have been ascribed to the considerable species and vessel heterogeneity in EDHF responses and the possibility that there might be more than one EDHF.

In particular, of the above candidates, there has been considerable support for a role for CYP metabolites. However, this hypothesis has come under severe scrutiny, and the observations in this issue of Clinical Science by Passauer et al. [10] add further to the growing belief that an EET is not a candidate for a ubiquitous EDHF.

EETs are synthesized from arachidonic acid by CYP epoxygenases, with the CYP 2C [11] isoform being the enzyme in the endothelial cell thought to be an EDHF synthase. Numerous reports have documented the efficacy of non-isoform-selective CYP inhibitors, such as clotrimazole and miconazole, in the blockade of EDHF responses in coronary arteries of several species, including humans [12]. However, this theory is controversial not least because CYP inhibitors have non-specific actions, including blockade of K+ channels [13,14], and in many cases the effects of EDHF cannot always be mimicked by metabolites of this pathway [15]. In addition,

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although the CYP inhibitors show functional blockade of EDHF responses in coronary arteries of some species, this does not seem to be the case in most other artery types [2].

However, recently an alternative hypothesis with respect to the role of EETs in EDHF responses has been proposed. More recent evidence suggests that in many (and probably most) arteries, EETs, rather than being EDHF, play a role in the pathway resulting in the release of EDHF. In particular, it has been postulated that EETs act in an autocrine fashion in the endothelial cell to establish sufficient elevation of endothelial intracellular \( [\text{Ca}^{2+}] \) to activate the hyperpolarizing pathways obligatory for the generation of an EDHF response [2,16]. These studies have all been conducted in cell types and tissues other than human and, since it is already clear that considerable heterogeneity exists not only between tissues, but also species, it is essential that they are repeated in humans tissues. As the momentum in the EDHF field has increased, especially with the observations that EDHF may have a compensatory role in inflammatory cardiovascular disease, studies in human tissues have emerged. In both isolated human arteries and \textit{in vivo} (human forearm flow studies), it is clear that an NO/PGI\(_2\)-independent vasodilation exists in response to endothelium-dependent vasodilators, such as acetylcholine or bradykinin. Moreover, this vasodilation is sensitive to blockers, albeit in some cases in a non-selective manner, of the endothelial and VSM hyperpolarizing pathways of EDHF responses [9,17,18]. The obvious and testable question that naturally follows is what does a CYP inhibitor do in these assays? There are several examples demonstrating that CYP inhibitors attenuate EDHF responses in isolated human arteries (e.g [19,20]). Similarly, in healthy human subjects following oral aspirin and local \textit{l-}NMMA infusion, bradykinin has been shown to cause a vasodilation that is partially suppressed by co-infusion with miconazole [18]. However, in contrast, Passauer et al. [10] now demonstrate in an almost replicate \textit{in vivo} system that bradykinin-induced vasodilation is unaffected by the CYP2C-selective inhibitor sulphaphenazole. As the authors [10] quite rightly point out, the discrepancies between these studies is most likely due to the fact that sulphaphenazole, unlike miconazole, does not exhibit some of the non-specificity characteristics of most CYP inhibitors. Therefore it is likely that the findings of Passauer et al. [10] most accurately describe the effects of CYP inhibition in the human forearm with respect to EDHF.

So what does this all mean in terms of the role of EETs in EDHF responses? Well, it is uncertain. Passauer et al. [10] clearly demonstrate no role for CYP metabolites in the human forearm either in basal flow or stimulated flow conditions. However, although this precludes a role for an EET in this vascular bed, it is possible that in the coronary vasculature, at least, sulphaphenazole would display profound EDHF-suppressing activity. Furthermore, as the authors [10] point out in patients with hypertension, for example, where EDHF responses appear to be up-regulated, EETs may play a role in endothelium-dependent vasodilation, since it is clear that CYP activity goes up in cardiovascular disease [12]. Whether this is the case or not, the study by Passauer et al. [10] highlights that, as with all other experimental species, EDHF displays remarkable heterogeneity between different vascular beds, unlike its counterpart endothelium-derived releasing factor (EDRF), which is ubiquitously NO. Rather than being a bad thing, this may be a valuable difference that can be exploited therapeutically. The problem with NO donors and NOS inhibitors is their inability to differentiate between vascular beds; these drugs will produce increases or decrease in vascular flow in all vascular beds to varying degrees. In contrast, the heterogeneity of EDHF intimates that modulators of EDHF pathways might allow regional changes in blood flow and, therefore, limited systemic activity.

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REFERENCES

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