Endothelium-Derived Hyperpolarizing Factor in the Brain
A New Regulator of Cerebral Blood Flow?

Elke M. Golding, PhD; Sean P. Marrelli, PhD; Junping You, MD, PhD; Robert M. Bryan, Jr, PhD

EDHF Defined
It is well established that stimulation of receptors on the endothelium can elicit dilation of arteries and arterioles by initiating the synthesis and release of nitric oxide (NO) and/or metabolites of the cyclooxygenase pathway (most often prostacyclin). Recent evidence now suggests that there is at least one other endothelium-dependent dilator mechanism that does not involve NO or a cyclooxygenase metabolite. This mechanism has been termed “endothelium-derived hyperpolarizing factor” (EDHF). (Note that EDHF is different from endothelium-derived relaxing factor [EDRF], which is often associated with NO. It should also be noted that recent studies suggest that EDHF may not be a “factor” but rather a process or mechanism. To be more accurate, the term “EDHF” should be referred to as a non-NO, noncyclooxygenase endothelium-dependent hyperpolarization. However, in order to maintain consistency in the literature, we will refer to it as “EDHF.”) We believe that EDHF is a major regulator of cerebral blood flow during physiological states and may become even more important following pathological insults such as ischemia or traumatic brain injury. The purpose of this editorial is to familiarize the reader with EDHF and to highlight the potential importance of EDHF in the cerebral circulation.

While the defining criteria for EDHF or the mechanism of endothelium-dependent hyperpolarizations can vary, we will characterize the process as (1) requiring endothelium, (2) being distinct from NO and a cyclooxygenase metabolite, (3) hyperpolarizing the vascular smooth muscle (VSM), and (4) involving calcium-activated potassium (KCa) channels.

EDHF in the Periphery
The existence of a third pathway was alluded to in the late 1980s, when some endothelium-dependent relaxations were shown to be resistant to inhibitors of NO and cyclooxygenase.1,2 Although it has been suggested that EDHF-dependent dilations reflect incomplete inhibition of nitric oxide synthase (NOS),3 there is now convincing evidence of the existence of a non-NO, noncyclooxygenase pathway. In cerebral vessels, extensive studies with a combination of inhibitors4 and eNOS knockout mice5 demonstrate that a pathway independent from NO is involved. These relaxations were further characterized by hyperpolarization of the VSM and sensitivity to some inhibitors of KCa channels. The VSM hyperpolarization serves to close voltage-gated calcium channels, resulting in decreased concentrations of cytoplasmic Ca2+ and ultimately VSM relaxation (or dilation).

Over the past 10 years, there has been much debate as to the mechanism associated with the EDHF-mediated dilations. The first idea involved epoxyeicosatrienoic acids (EETs), a product of arachidonic acid metabolism through the cytochrome P450 epoxygenase pathway.6–8 EETs, which are either stored in the endothelium or synthesized on demand, were proposed to diffuse to the VSM, open KCa channels, and hyperpolarize the VSM.9

The second idea is that the potassium ion (K+) is EDHF.10 On stimulation of endothelial receptors, the K+ conductance of endothelial cells would increase by opening KCa channels. This would be followed by an efflux of K+ from endothelial cells as a result of the electrochemical gradient causing K+ to increase from 3 mmol/L to ~12 mmol/L in the extracellular space between the endothelial and VSM cells.10 The increase in potassium would hyperpolarize the VSM by activating inwardly rectifying potassium channels and the Na+/K+ pump. As stated above, the hyperpolarization would then lead to dilation.

A third line of studies implicate gap junctions that couple endothelial cells and VSM cells (myoendothelial gap junctions). These myoendothelial junctions would provide a conduit for either the EDHF or an electrical current to move between the endothelium and VSM.11,12 If the process involves only the movement of current, then “EDHF” would not be a factor per se but rather a process. Consequently, “endothelium-dependent hyperpolarization” might best describe the phenomenon.

Additionally, there is evidence that hydrogen peroxide,12 anandamide,13 or products of the lipoxygenase pathway14,15 are involved with the EDHF dilations. Although these latter mediators have been less studied and, therefore, less scrutinized, they may be no less significant. In all likelihood, several EDHFs and/or EDHF-like processes exist and are dependent on the vascular bed, species, and physiological state. For more in-depth reviews of the EDHF mechanisms in peripheral vessels see the previous works by Feletou and Vanhoutte16 and McGuire et al.17

EDHF in the Brain
Although it was previously known that cerebrovascular smooth muscle could be hyperpolarized by endothelial mechanisms,18 it was not until 1995 that evidence began to emerge.
for the existence of EDHF in cerebral vessels. ATP, UTP, substance P, A23187 (Ca$^{2+}$ ionophore), and acetylcholine have been reported to elicit dilations through the release of EDHF in addition to NO.\textsuperscript{4,19–29} Of significance, the EDHF response was shown to exist in human cerebral arteries.\textsuperscript{19} The dilator mechanism in these aforementioned studies fits the criteria for EDHF. That is, it required intact endothelium, it was not blocked by inhibitors of NO synthase or cyclooxygenase, it hyperpolarized the VSM by $\approx$15 mV, and it was blocked by inhibitors of K$_{Ca}$ channels.

Very little is known regarding the identity of EDHF or mechanism of action in cerebral vessels. Ca$^{2+}$ does appear to be a second messenger in the pathway, with the amount of endothelial Ca$^{2+}$ required to elicit an EDHF-mediated dilation being approximately 100 nmol/L greater than that required to elicit a purely NO-mediated dilation (340 nmol/L versus 220 nmol/L, respectively).\textsuperscript{50} Most significantly, the EDHF response in cerebral vessels appears to be distinctly unique; that is, the identity and/or process of EDHF in cerebral vessels is likely different from that in peripheral vessels. This is illustrated by the work of Dong and coworkers, who reported that while clotrimazole attenuated EDHF-mediated responses in the guinea pig cerebral artery, it had no effect on the mesenteric artery.\textsuperscript{27} Furthermore, while studies in the periphery have demonstrated that basal levels of NO inhibit EDHF,\textsuperscript{31} this was not shown to be the case in cerebral arteries.\textsuperscript{32} Therefore, the EDHF mechanism cannot be directly extrapolated from the mechanism in peripheral vessels, and it is imperative that EDHF is identified in cerebral vessels independently of peripheral vessels.

The significance of EDHF in the brain is reflected in 3 recent observations:

1. **The Relative Contribution of EDHF Increases as Vessel Caliber Decreases**

The EDHF response elicited by the luminal application of ATP has been shown to play a more prominent role in third- and fourth-order branches of the middle cerebral artery and penetrating arterioles than in middle cerebral arteries.\textsuperscript{25,26} This was in direct contrast to NO, which showed a diminishing role along the cerebrovascular tree.\textsuperscript{25} These findings suggest that with respect to purine and pyrimidine nucleotide stimulation, EDHF may be the predominant dilator mechanism in the regulation of smaller resistance arteries and arterioles. Due to the fundamental role of these smaller vessels in the control of vascular resistance, it would therefore appear that EDHF plays an important role in the regulation of vascular resistance and, thus, in the control of cerebral blood flow during normal physiological states.

2. **EDHF Is Upregulated Following Pathological Conditions**

A potentiation of the EDHF response has been observed following ischemia reperfusion injury\textsuperscript{25,28} and traumatic brain injury.\textsuperscript{26,33} The EDHF component of the ATP dilation was shown to be enhanced in pial arterioles at 24 hours but not 1 hour following mild traumatic brain injury.\textsuperscript{33} Interestingly, the upregulation of EDHF following ischemia/reperfusion more than compensated for a suppressed response to NO that also occurred.\textsuperscript{23} While there is still much to be learned about the upregulation of EDHF following these pathological con-

ditions, we presently speculate that it is a protective mechanism that helps to preserve endothelial-dependent relaxation of the injured cerebral arteries. Of note, we do not know if the upregulated response is the same or a different “EDHF” response to that occurring during nonpathological states.

3. **EDHF Is Downregulated by Estrogen**

Unlike vessels isolated from male rats, the response to ATP in female middle cerebral arteries showed a negligible EDHF component of the dilation.\textsuperscript{34} Moreover, the contributing factor was estrogen, since EDHF-mediated dilations showed major enhancement in vessels isolated from ovariectomized females, and this upregulated EDHF response was again lost following estrogen replacement. This same effect of estrogen on the EDHF response was demonstrated in vivo using the pial window technique.\textsuperscript{35} Interestingly, the effects of estrogen on the EDHF response in cerebral vessels are just the opposite of those reported in peripheral vessels. Estrogen upregulates the EDHF response in peripheral vessels.\textsuperscript{36} Again, the response to estrogen provides more evidence that the EDHF response in cerebral vessels is unique. The implications of estrogen’s effect on EDHF in brain have yet to be determined, particularly in light of the fact that estrogen has displayed a protective influence following stroke.\textsuperscript{37,38}

**Does EDHF Play a Role In Vivo?**

Most studies of EDHF to date have been carried out on isolated vessels. Measurement of VSM hyperpolarization, one of the criteria defining EDHF, cannot be measured practically in cerebral arteries and arterioles in vivo. Nevertheless, dilations in pial vessels in vivo that are resistant to inhibitors of NO synthase and cyclooxygenase have been observed,\textsuperscript{5,35} and these dilations fit the criteria for EDHF with the exception of VSM hyperpolarization. Whether these dilations involve the EDHF response or not is irrelevant, because the observations would still represent a third endothelium-dependent mechanism of dilation in cerebral vessels. Notwithstanding the technical limitations, evidence is emerging that the EDHF response or a similar response does exist in vivo.\textsuperscript{5,35}

**What Does the Future Hold?**

The discovery of a new endothelial-mediated dilatory process is intriguing, but many questions must be answered before EDHF can be considered a bona fide regulator of cerebral blood flow. For example, what is its physiological role? When is it functional? Does it play a role in vivo during pathological states? Presently, EDHF is primarily studied following inhibition of both NO and the cyclooxygenase pathway. Only when the mechanism of EDHF-mediated dilations is elucidated can it be studied in the presence of NO and cyclooxygenase, and only then can its relative importance in cerebral vessels during normal physiological conditions be determined. Furthermore, interpretation of these mechanisms is complicated by the fact that K$_{Ca}$ channel inhibitors can inhibit both NO and EDHF. Nevertheless, we believe that the existing studies are provocative and indicate the necessity for further investigations involving EDHF in the brain. If EDHF does indeed prove to be protective following pathological conditions, it will be a powerful addition to our understanding of cerebral hemodynamics.
conditions, as we speculate, then it must be considered as a therapeutic strategy to be used in the clinical treatment of brain injury. The challenge for the future lies not only in the characterization of EDHF but also in its role in regulating cerebral blood flow.

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