

PERSPECTIVES

EDHF – are there gaps in the pathway?

G. Edwards and A. H. Weston

School of Biological Sciences, University of Manchester, Manchester M13 9PT, UK

Vascular endothelial cells release a variety of vasorelaxant factors in response to stretch or to agonists. Attention has mainly focused on two such factors, prostacyclin and nitric oxide, the products of the enzymes cyclo-oxygenase and nitric oxide synthase, respectively. However, when these enzymes are inhibited, most blood vessels still exhibit endothelium-dependent relaxations when exposed to ligands such as acetylcholine, substance P and bradykinin. These dilatations are associated with smooth muscle hyperpolarization characteristic of K^+ channel opening and the term 'endothelium-derived hyperpolarizing factor' (EDHF) was coined to describe the putative endothelial factor released (Taylor & Weston, 1988).

A breakthrough occurred when Waldron & Garland (1994) and Zygmunt & Högestätt (1996) showed that EDHF responses were inhibited by the simultaneous presence of apamin and charybdotoxin but not by either toxin alone. Apamin selectively inhibits small conductance Ca^{2+} -sensitive K^+ (SK_{Ca}) channels while charybdotoxin blocks several types of K^+ channel, including intermediate- and large conductance Ca^{2+} -sensitive K^+ channels (IK_{Ca} and BK_{Ca} , respectively) which are present in the vasculature. However, in the presence of apamin, iberiotoxin (a selective inhibitor of BK_{Ca}) does not inhibit the smooth muscle EDHF response (Zygmunt & Högestätt, 1996). Collectively, this pharmacological profile has become a defining feature of the EDHF pathway.

At the time of these toxin studies, it was widely assumed that the charybdotoxin- and apamin-sensitive channels were present on the smooth muscle cells. Working independently, Marchenko & Sage (1996) showed that two Ca^{2+} -sensitive K^+ channels of different unitary conductance were present on the endothelial cells of rat aorta. These findings, together with those of Waldron & Garland (1994) and of Zygmunt & Högestätt (1996), led Edwards & Weston (1998) to speculate that the actions of the toxin inhibitors were exerted on Ca^{2+} -sensitive K^+ channels located on the endothelium and not on the smooth muscle. Subsequently, using rat hepatic and mesenteric arteries in a combined micro-electrode and myograph study, strong evidence was obtained that the inhibitory effects of charybdotoxin and apamin were indeed

exerted on the vascular endothelium (Edwards *et al.* 1998).

There are several endothelium-derived factors which are capable of hyperpolarizing the surrounding smooth muscle. Two of these – nitric oxide and epoxyeicosatrienoic acids such as 11,12-EET – open smooth muscle BK_{Ca} channels and their actions are inhibited by iberiotoxin. Prostacyclin, a third factor, opens the ATP-sensitive K^+ channel, an action which is glibenclamide sensitive (see Edwards & Weston, 1998). Although all of these are 'endothelium-derived hyperpolarizing factors', the acronym 'EDHF' is correctly applied only to that factor, the response (hyperpolarization or relaxation) to which is observed in the presence of cyclo-oxygenase plus nitric oxide synthase inhibitors and which is inhibited by charybdotoxin + apamin but unaffected by iberiotoxin + apamin.

The identity of EDHF is the subject of heated debate. An EET is the candidate favoured by some groups (Campbell *et al.* 1996; Fisslthaler *et al.* 2000) and the actions of 11,12-EET have been widely studied, especially in porcine coronary arteries. Although this eicosanoid is an endothelium-derived hyperpolarizing factor in this vessel, its action is iberiotoxin sensitive whereas the EDHF pathway in this vessel is iberiotoxin insensitive (Edwards *et al.* 2000). An alternative proposal is that EDHF is endothelium-derived K^+ . In their studies on rat hepatic and mesenteric arteries, Edwards *et al.* (1998) showed that K^+ effluxing from the vascular endothelium via charybdotoxin- and apamin-sensitive K^+ channels could hyperpolarize and relax the surrounding smooth muscle by stimulating the smooth muscle Na^+, K^+ -ATPase and inwardly rectifying K^+ channels. Although such a mechanism, in which K^+ can be considered to be EDHF, can largely explain the response in these vessels (Edwards *et al.* 1998; Dora & Garland, 2001), ' K^+ -coupling' cannot explain the findings in the guinea-pig internal carotid and porcine coronary arteries (Quignard *et al.* 1999; Edwards *et al.* 2000). In these and certain other arteries, endothelial hyperpolarization may be transferred electrotonically to the smooth muscle via myo-endothelial gap junctions, a model proposed by Chaytor *et al.* (1998).

In this issue of *The Journal of Physiology*, Coleman *et al.* have examined the potential role of K^+ in the EDHF pathways in guinea-pig submucosal, human subcutaneous and guinea-pig coronary arteries. In a micro-electrode, patch clamp and myograph study of the highest quality and of supreme technical difficulty, the authors elicited EDHF effects with acetylcholine in these

vessels under conditions in which elevation of extracellular K^+ produced different or no effects. In the rat hepatic artery, however, they did observe EDHF-like responses following elevation of extracellular K^+ .

Coleman *et al.* (2001) clearly show that a high degree of coupling exists between the endothelial and smooth muscle layers in some mammalian arterioles. They conclude that gap junctions play a key role in the EDHF pathway in guinea-pig submucosal, human subcutaneous and guinea-pig coronary arteries and their data even question the existence of a hyperpolarizing factor *per se* in these vessels. Definitive proof of their conclusions was prevented by the lack of a selective gap junction inhibitor and their study highlights the urgent need for such agents. Ideally such inhibitors would target only myo-endothelial gap junctions and not those coupling endothelial and smooth muscle cells within their respective layers.

Study of the EDHF phenomenon has brought sharp microelectrode techniques back into fashion. It was Edith Bülbring who pioneered this methodology for smooth muscle and who published many of her findings in *The Journal of Physiology*. She would surely be delighted to know that her priceless legacy lives on.

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