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Endothelial potassium channels, endothelium-dependent hyperpolarization, and the regulation of vascular tone in health and in disease

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Summary

1. The elusive nature of endothelium-derived hyperpolarizing factor (EDHF) has hampered detailed study of the ionic mechanisms that underlie the EDHF hyperpolarization and relaxation. Most studies have relied on a pharmacological approach in which interpretations of results can be confounded by limited specificity of action of the drugs used. Nevertheless, small-, intermediate-, and large- conductance Ca^{2+}-activated K^+ channels (SK_{Ca}, IK_{Ca}, and BK_{Ca}, respectively), have been implicated, with inward rectifier K^+ channels (K_{IR}) and Na^+/K^+ ATPase also suggested by some studies.

2. Endothelium-dependent membrane currents recorded using single electrode voltage-clamp from electrically short lengths of arterioles in which the smooth muscle and endothelial cells remained in their normal functional relationship have provided useful insights into the mechanisms mediating EDHF. Charybdotoxin (ChTx) or apamin reduced, while apamin plus ChTx abolished the EDHF current. The ChTx and apamin sensitive currents both reversed near the expected K^+ equilibrium potential, were weakly outwardly rectifying, and displayed little, if any, time or voltage-dependent gating, thus having the biophysical and pharmacological characteristics of IK_{Ca} and SK_{Ca} channels, respectively.

3. IK_{Ca} and SK_{Ca} channels occur in abundance in endothelial cells and their activation results in EDHF-like hyperpolarization of these cells. There is little evidence for a significant number of these channels in healthy, contractile vascular smooth muscle cells.

4. In a number of blood vessels in which EDHF occurs, the endothelial and smooth muscle cells are electrically coupled via myoendothelial gap junctions. In contrast, in the adult rat femoral artery, in which the smooth muscle and endothelial layers are not coupled electrically, EDHF does not occur, even though acetylcholine evokes hyperpolarization in the endothelial cells.

5. *In vivo* studies indicate that EDHF contributes little to basal conductance of the vasculature, but it contributes appreciably to evoked increases in conductance.

6. EDHF responses are diminished in some diseases including hypertension, preeclampsia and some models of diabetes.

7. The most economical explanation for EDHF *in vitro* and *in vivo* in small vessels is that it arises from activation of IK_{Ca} and SK_{Ca} channels in endothelial cells. The resulting endothelial hyperpolarization spreads via myoendothelial junctions to result in the EDHF-attributed hyperpolarization and relaxation of the smooth muscle.

Introduction

Endothelial K^+ channels have been widely implicated in endothelium-dependent vasodilation. Initially it was considered that endothelial cell hyperpolarization, via the opening of K^+ channels, would facilitate Ca^{2+} influx in these cells by increasing the driving force for this cation[1,2] and in this way enhance production of the "classical" endothelium-dependent vasorelaxants NO and PGI_2, which rely on an increase in cytoplasmic free Ca^{2+}. However, since the Ca^{2+} equilibrium potential is likely to be around +130 mV, a large driving force of +190 mV for Ca^{2+} influx exists at a resting potential of −60 mV. This means that endothelial hyperpolarization would be expected to contribute little extra to the driving force for Ca^{2+} influx. Under such conditions, block of endothelial hyperpolarization might be expected to have little effect on cytoplasmic Ca^{2+} levels. Such has been shown to be the case[3,4].

The discovery of the additional vasodilator phenomenon of endothelium-derived hyperpolarizing factor (EDHF) has prompted renewed interest in the role of endothelial K^+ channels in the regulation of vascular tone. EDHF is so-called because its vasodilator effects are strongly associated with smooth muscle hyperpolarization, and because the nature of EDHF was unknown[5-7] and remains controversial[8,9]. There are currently three main suggestions as to the nature of EDHF, which are not mutually exclusive but may represent differences between species, between vascular beds and between different endothelial stimulants. One suggestion is that EDHF represents endothelial hyperpolarization generated by the activation of Ca^{2+}-activated K^+ channels (K_{Ca}) that spreads passively via myoendothelial gap junctions to result in hyperpolarization of the smooth muscle cells[10-17]. According to this idea, endothelial K^+ channels would influence smooth muscle contractile activity by reducing Ca^{2+} influx via voltage-operated Ca^{2+} channels and by suppression of key enzymes involved in agonist-induced transduction pathways[18,19]. Another suggestion is that EDHF is a product of the cytochrome P450 pathway, such as an epoxyeicosatrienoic acid (EET), and since EETs can activate large-conductance, Ca^{2+}-activated K^+ channels (BK_{Ca}), it has been inferred that EDHF evokes hyperpolarization via the activation of BK_{Ca} channels on the smooth muscle cells[20-27]. The third suggestion is that K^+ efflux from endothelial cells via intermediate- and small-conductance Ca^{2+}-activated K^+ channels (IK_{Ca} and

SK_{Ca}, respectively), activates inward rectifier K^+ channels (K_{IR}) and the Na^+/K^+ATPase on the smooth muscle cells[28]. Thus, different ionic mechanisms have been proposed to underlie the actions of EDHF. EDHF plays an increasingly prominent role in vasodilation as arterial diameter decreases, and is thus likely to be important in tissue perfusion. Since EDHF appears to decline with advancing age and to be targeted in diseases such as hypertension and diabetes, knowledge of the ionic mechanisms underlying EDHF would be expected to give an improved understanding of the nature of EDHF and to impact on our understanding of the regulation of vascular tone in health and in disease, and this will be the focus of the present article.

Pharmacology of EDHF relaxation and hyperpolarization

Earliest studies to identify the ionic mechanisms underlying EDHF utilized blockers of various ion pathways. Of concern was that the effects observed could have resulted from an action of the drugs used on the endothelial cells, thus affecting the production of EDHF, rather than the EDHF response in the smooth muscle. Early studies demonstrated an efflux of ^{86}Rb[5], an increase in membrane conductance[29], and an insensitivity to the Na^+/K^+ATPase inhibitor ouabain[30] which suggested that EDHF activates a K^+ conductance. The K^+ channel blockers apamin (selective for SK_{Ca} channels)[31] or charybdotoxin (ChTx, which blocks BK_{Ca}, IK_{Ca}, and some voltage-dependent K^+ channels, K_V)[32] abolished EDHF relaxations, but in other studies, either blocker by itself had little, if any, effect. However, total block was achieved by a combination of apamin plus ChTx[4,33-39]. A general lack of effectiveness of blockers of K_{ATP} and K_V channels indicated that these channels were unlikely to be involved[31,33-35,40]. Iberiotoxin (IbTx), which selectively blocks BK_{Ca} channels, inhibited the EDHF relaxation in some studies *in vivo*[41] and *in vitro*[42,43] but was ineffective in other studies against the EDHF relaxation[34,35,44-46] or hyperpolarization[26,45,47]. This ineffectiveness of IbTx, together with at least partial block by ChTx, suggested that the ChTx-sensitive channel was the IK_{Ca} channel[44]. Although tetraethylammonium (TEA, which blocks BK_{Ca} and some K_V channels) produced an effect in some studies[32,35,44], the anti-muscarinic actions of TEA[48] may cloud the interpretation of its effects. 4-Aminopyridine (4-AP, which blocks K_V channels) diminished the EDHF response in some studies, but an alternative explanation is that it did so through inhibition of the increase in endothelial cytoplasmic free Ca^{2+} [4].

In electrophysiological studies, K_V and K_{ATP} blockers did not affect the EDHF hyperpolarization in the guinea-pig coronary artery[45,49-51]. However, the hyperpolarization was reduced by TEA (1-5mM), ChTx (5×10^{-8} M) and 4-AP[49-51], while apamin had no effect[45,49] or caused a small reduction in the initial phase of the hyperpolarization[51]. Somewhat similarly, in guinea-pig carotid arteries and submucosal arterioles, the EDHF hyperpolarization was insensitive to blockers of K_{ATP} and K_V channels, but was reduced by ChTx and further reduced by ChTx plus apamin[52-54]. In the rat, the EDHF hyperpolarization in the tail artery was abolished by a combination of ChTx plus apamin[55], while in the mesenteric artery, apamin was more effective than ChTx, but both were required to completely block the EDHF hyperpolarization and relaxation[56]. In the mesenteric artery of the rabbit, apamin alone abolished the EDHF hyperpolarization, as did TEA (10mM), while it was unaffected by ouabain, 4-AP, or Ba^{2+} [57].

Overall, the studies on EDHF-induced hyperpolarizations and relaxations produced no strong evidence for the involvement of K_V or K_{ATP} channels, evidence for the involvement of BK_{Ca} channels in several studies, and strongly implicated IK_{Ca}, and SK_{Ca} channels in many other studies. More recently, selective and potent blockers of IK_{Ca} channels have been developed that are analogues of clotrimazole that lack the imidazole ring and therefore do not block cytochrome P450 enzymes[58]. These compounds, TRAM-34 and TRAM-39, particularly in combination with apamin, block the EDHF hyperpolarization and relaxation, providing stronger pharmacological evidence for the involvement of IK_{Ca} channels, in addition to SK_{Ca} channels[59-62].

K^+ as an EDHF

The elegant hypothesis that EDHF may be none other than K^+ released from the endothelial cells raised additional candidates for the ionic mechanisms underlying EDHF[28]. According to this scheme, stimulation of endothelial cells results in the activation of endothelial K_{Ca} channels. The resulting efflux of K^+ is then proposed to accumulate in the myoendothelial space where it stimulates the Na^+/K^+ ATPase and K_{IR} channels in the smooth muscle[28]. This study gave a fresh boost to investigations into the ionic mechanisms underlying the EDHF hyperpolarization. Using low concentrations of Ba^{2+} to specifically block K_{IR} (typically around 30 μM), ouabain to block the Na^+/K^+ ATPase, and attempted mimicry by the exogenous application of modest increases in KCl, a number of studies obtained evidence against the K^+ hypothesis[63-67], while other studies provided evidence in favour of the idea[38,39,68-70]. Such studies have generally placed strong emphasis on block of EDHF responses by ouabain. However, the effects of ouabain need to be interpreted with considerable caution. Ca^{2+} overload[71-73] has been invoked to explain an inhibition of a K^+ channel by a 10 minute exposure to ouabain in canine ventricular myocytes[74], while ouabain also inhibited the iloprost-induced hyperpolarization, which is inhibited by glibenclamide, in the rat hepatic artery[16]. In the bovine coronary artery, ouabain blocked relaxations induced by the NO donor glyceryl trinitrate[39]. A recent study indicating that ouabain is capable of decreasing gap junction permeability[75] is particularly significant since such effects are consistent with EDHF being due to electrotonic spread of hyperpolarizing current from the endothelium to the smooth muscle (see below). In that study, the cells were exposed to ouabain for one hour, which is appreciably longer than in studies on the

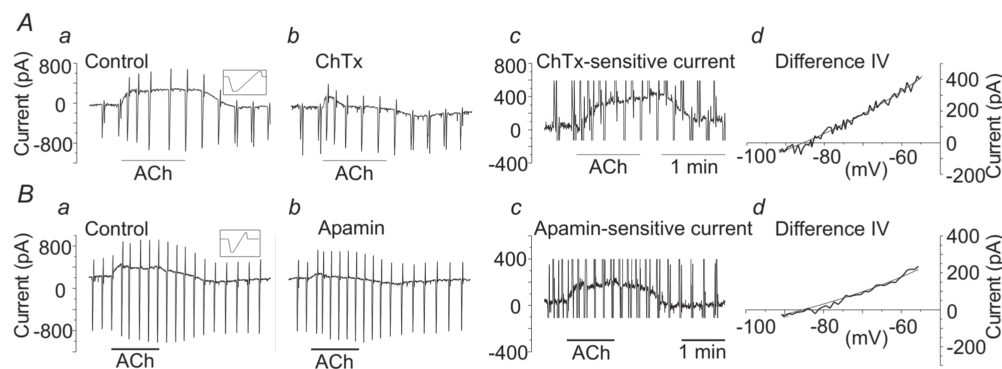

Figure 1. Components of EDHF current recorded from segments of guinea-pig submucosal arterioles.
Aa, Ba, ACh (1 μM) evoked an outward, EDHF current with the membrane clamped at –63 mV. Periodic transients are responses to voltage ramps (insets). Ab, ChTx (30 nM) and Bb, apamin (0.5 μM) reduced the EDHF current. Ac, Bc, subtraction of the current in Ab from Aa reveals the ChTx-sensitive component of current, and subtraction of the current in Bb from Ba reveals the apamin-sensitive component of current. Ad, Bd, the I-V relationships for the ChTx-sensitive and apamin-sensitive components, respectively, were well-described by the GHK equation for a K^+ current (smooth lines). Reproduced with permission of The Physiological Society from Coleman et al[16].

effects of ouabain on EDHF. The effects of shorter duration exposures to ouabain on gap junction permeability were not determined.

Voltage-clamp studies

Ionic mechanisms are perhaps ideally studied by recording the membrane currents under voltage-clamp. Voltage-clamp studies of vascular tissues typically involve enzymatic isolation of either the smooth muscle or endothelial cells, and recording from the isolated cells using the patch-clamp technique. Such cellular isolation overcomes the problems of spatial clamp control in a syncytial tissue. However, to record the ionic currents underlying the elusive and controversial EDHF, a preparation was required in which the endothelial and smooth muscle cells remained in their normal functional relationship, especially in view of electrotonic spread as a potential mechanism. Such a preparation needed to be amenable to voltage-clamp, preferably without exposing the cells to digestive enzymes that could potentially disrupt mechanisms underlying EDHF. Hirst and Neild[76] demonstrated that the submucosal arterioles lying in the wall of the small intestine of the guinea-pig had an electrical length constant of about 1600 μm, and that the arterioles could be cut into short segments that remained physiologically viable. Hirst and colleagues subsequently showed that if the arterioles were cut into sufficiently short lengths, they could be voltage-clamped with a single intracellular microelectrode using a switching amplifier[77], though the limited current-passing ability of the microelectrodes restricted the range of potentials over which the membrane could be clamped. The contractile activity of these arterioles could also be recorded using the video tracking hardware and software of diamtrak, developed by Neild [78]. These arterioles therefore seemed a good preparation in which to record the EDHF currents under voltage-clamp, and also to determine their functional significance in terms of contractile activity. However, it must be borne in mind that increasing the amount of stretch in the wall of the guinea-pig coronary artery increased the amplitude of hyperpolarization evoked by NO, iloprost, and EDHF, though the EDHF hyperpolarization was less sensitive to stretch than that of NO and iloprost[79]. Thus, since the short segments of arterioles cannot be pressurized, there may be some differences in the activity of the underlying ion channels and their regulatory mechanisms compared with the more physiological, pressurized state, in which the ionic mechanisms cannot be readily studied.

In the submucosal arterioles, with the membrane potential clamped at around –65mV, and in the presence of N^{ω}-nitro-L-arginine methylester (L-NAME) and indomethacin to inhibit NO production and cyclooxygenase activity, respectively, acetylcholine (ACh) and substance P evoked an outward current attributed to EDHF[16,17] (Fig 1Aa, Ba) and also resulted in EDHF-induced relaxation[16,17]. Current-voltage (I-V) relationships, obtained from the current responses to periodic voltage ramps, revealed that the EDHF current reversed at a potential around that for K^+, indicating that the EDHF current involved the activation of K^+ channels. ChTx reduced the EDHF current (Fig 1Ab), and by subtraction of currents, the ChTx-sensitive component was revealed (Fig 1Ac). Its I-V relationship was well described by the Goldman-Hodgkin-Katz (GHK) equation for a K^+ current (Fig 1Ad), indicating that the ChTx-sensitive component of current involved the activation of K^+ channels whose gating was insensitive to membrane potential. This voltage-insensitivity, together with block by ChTx but not IbTx, provides both biophysical and pharmacological evidence that this component of

current was carried by IK_{Ca} channels[16]. Apamin similarly inhibited a component of current (Fig 1Bb,c) whose I-V relationship was well-described by the GHK equation for a K^+ current (Fig 1Bd). An insensitivity to gating by membrane potential, together with block by apamin, indicates that this component of current was carried by SK_{Ca} channels. In the combined presence of ChTx plus apamin, the EDHF current and relaxation were abolished, indicating that the only currents contributing to the EDHF response were those flowing through IK_{Ca} and SK_{Ca} channels in this preparation[16].

Ba^{2+} inhibited a component of the holding current whose I-V relationship was inwardly rectifying, typical of K_{IR} channels, and very different to the I-V curves for the EDHF components of current[16,17] (Fig 2). Ouabain also inhibited a component of the holding current, and its I-V relationship was typical of that for the Na^+/K^+ATPase, and very different to that for the EDHF currents[16] (Fig 2). The addition of 5 - 10 mM KCl activated a current which was largely blocked by Ba^{2+} [16,17]. These results indicate that K_{IR} channels and the Na^+/K^+ATPase contribute to the resting current in the submucosal arterioles, and that the K_{IR} channels can be activated by the addition of K^+. Significantly, however, these results provide strong evidence that K_{IR} channels and the Na^+/K^+ATPase do not contribute to the EDHF current in these arterioles.

Myoendothelial electrical coupling and the location of IK_{Ca} and SK_{Ca} channels

The involvement of IK_{Ca} and SK_{Ca} channels in the EDHF response raises the critical question of where these channels are located. An associated question is whether the endothelial and smooth muscle cells are electrically coupled, since it has been suggested that EDHF may represent electrotonic spread of hyperpolarization from the endothelium to the smooth muscle[14] (see above). Strong evidence indicates that such coupling occurs in a number of vessels (recently reviewed[80]). To test this possibility in guinea-pig submucosal arterioles, recordings of membrane potential were made from dye (Lucifer Yellow)-identified endothelial and smooth muscle cells. Excitatory junction potentials (EJPs) in response to sympathetic nerve stimulation, and action potentials associated with vasoconstriction, all of which were initiated in the smooth muscle cells, were also recorded from endothelial cells. Significantly, the responses recorded from the endothelial cells were indistinguishable from those recorded from the smooth muscle cells, indicating that the electrical coupling is very strong and that the two layers function essentially as a single electrical syncytium[16,17]. Such electrical coupling does not occur in all vessels. More recently, Sandow and colleagues found that in the more proximal parts of the adult rat femoral artery, there is a lack of both myoendothelial electrical coupling together with an absence of myoendothelial gap junctions[81]. Significantly, this lack of myoendothelial coupling was associated with a lack of EDHF-mediated hyperpolarization and relaxation in the smooth muscle, even though the endothelial cells hyperpolarized when stimulated with agents such as ACh and the hyperpolarization was blocked by ChTx plus apamin[81]. Furthermore, in the rat mesenteric artery, in which myoendothelial coupling is strong[81,82], use of connexin mimetics inhibited the EDHF response recorded from the smooth muscle but not the endothelial cell hyperpolarization. Caution is required in interpreting the effects of the connexin mimetics such as the Gap compounds since they must be used at relatively high concentrations, and there have been very few electrophysiological studies of their effects on electrical coupling. Nevertheless, taken as a whole, the observations of Sandow and colleagues[81] provide critical support for the idea that EDHF is generated in the endothelial cells and propagates via myoendothelial gap junctions to result in the smooth muscle EDHF hyperpolarization and relaxation.

An endothelial site for the initiation of the EDHF hyperpolarization suggests that the IK_{Ca} and SK_{Ca} channels are located in endothelial rather than in smooth muscle cells. Indeed, there is very little evidence that IK_{Ca} channels occur in normal, healthy, contractile smooth muscle cells, although electrophysiological and expression analysis reveal that IK_{Ca} channels can occur in cultured cells and during hyperplasia[83,84]. There is also little evidence that SK_{Ca} channels occur in non-cultured vascular smooth muscle cells[85,86]. In contrast, in endothelial cells, electrophysiology, immunohistochemistry, and expression analysis reveal an abundance of IK_{Ca} and SK_{Ca} channels[85-89]. Consistent with such observations, endothelial cells which are isolated and not in contact with vascular smooth muscles respond to ACh with hyperpolarization which can be reduced by ChTx[90,91] and abolished by ChTx plus apamin[81,91]. Furthermore, EDHF-induced relaxations of perfused mesenteric arteries were blocked when ChTx plus apamin were added to the perfusate in the lumen and thus applied selectively to the endothelial cells, but the relaxations were not blocked when these K^+ channel blockers were added to the superfusate[92].

EDHF *in vivo*

Despite numerous studies indicating that EDHF is capable of evoking considerable relaxation in small vessels *in vitro*, an important consideration is whether EDHF is functionally important *in vivo*. Significant relaxation *in vivo* has been reported for an EDHF response attributed to a product of the cytochrome P450 pathway[41,93-95] and blocked by IbTx, implicating BK_{Ca} channels[41]. This EDHF does not appear to contribute to basal tone *in vivo*[41]. The most widely reported EDHF response *in vitro* is that which is blocked by a combination of ChTx plus apamin and involves IK_{Ca} and SK_{Ca} channels located in the endothelium (discussed above). The *in vivo* significance of this form of EDHF was evaluated in the rat mesenteric and hindlimb beds[96]. In the presence of L-NAME and indomethacin, local infusion of ChTx plus apamin selectively into these beds had no effect on basal blood flow or conductance. However, these agents abolished the appreciable increases in blood flow and conductance evoked by ACh and

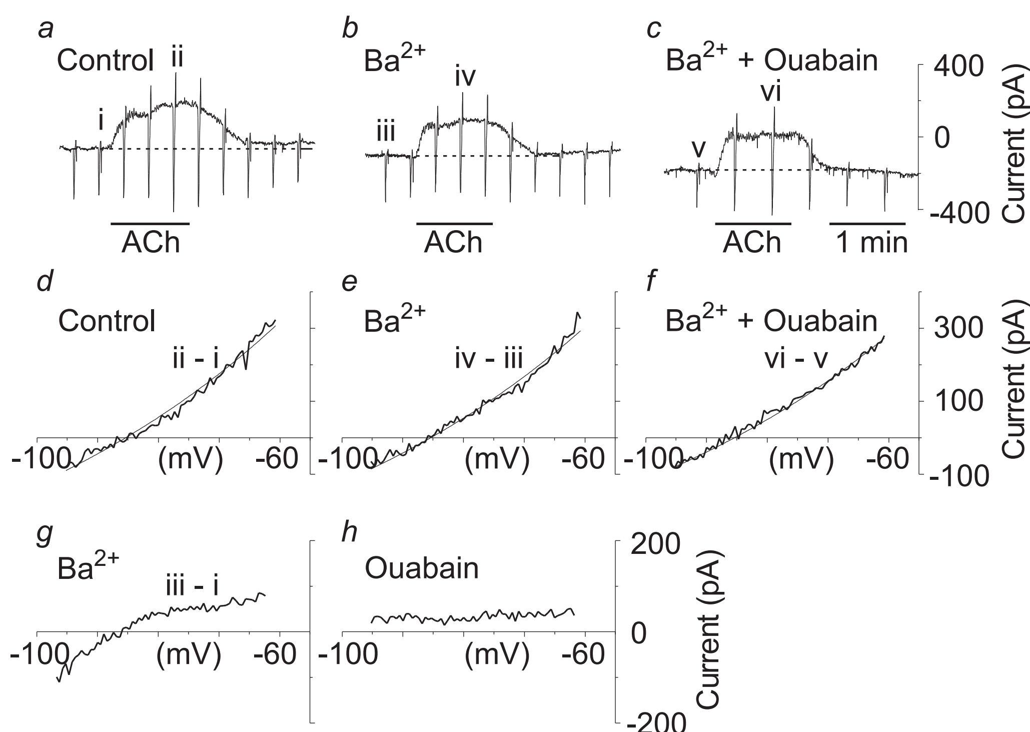

Figure 2. Contribution of K_{IR} and Na^+/K^+ATPase to arteriole currents
a, ACh (1 μM) evoked an outward, EDHF current. b, the EDHF current was not reduced by Ba^{2+} (30 μM), or c, by the addition of ouabain (200 μM) in the continuing presence of Ba^{2+}. d, the I-V relationship for EDHF obtained from the current responses to periodic voltage ramps in panel a, was well-described by the GHK equation for a K^+ current (smooth line), but was not affected by Ba^{2+} (e) or ouabain plus Ba^{2+} (f). g, Ba^{2+} inhibited a component of the holding current (b − a) which had an inwardly-rectifying I-V relationship typical of K_{IR} channels. h, ouabain inhibited a component of holding current (c − b) with a relatively flat I-V relationship typical of the Na^+/K^+ATPase. Reproduced with permission of The Physiological Society from Coleman et al*[16].*

bradykinin, whereas IbTx was ineffective. These results indicate that in these vascular beds, EDHF does not contribute to basal blood flow, but makes a significant contribution to evoked blood flow. These effects do not involve BK_{Ca} channels, but are due to activation of IK_{Ca} and SK_{Ca} K^+ channels located in the endothelial cells[96]. These results support and extend an earlier *in vivo* study in which connexin-mimetic peptides, thought to inhibit gap junctions, abolished EDHF-mediated increases in blood flow in the rat renal microcirculation[97].

EDHF in disease

Endothelial dysfunction is a feature of a number of diseases and this has prompted investigations into the fate of EDHF in various diseases. The effects of hypertension on EDHF have been assessed in vessels from spontaneously hypertensive rats (SHR) compared with vessels from Wistar-Kyoto (WKY) rats. In the mesenteric artery, the EDHF hyperpolarization was halved and the relaxation significantly reduced[98], while in the tail artery the hyperpolarization was decreased by 28%[55]. An increase in the number of layers of smooth muscle cells together with a greater incidence of myoendothelial gap junctions (MEGJs) in SHRs[55] might explain the decreased EDHF response in terms of an increased electrical "sink" for the endothelium-derived hyperpolarizing current. In preeclampsia, a pregnancy-specific form of hypertension in women, the EDHF vasodilator response in myometrial arteries is also significantly reduced and this may represent a failure of its up regulation as occurs in these tissues in the normal adaptation to pregnancy in healthy women[99] .

Changes in EDHF in diabetes have been studied in most detail in streptozotocin (STZ)- induced diabetes in rats. In the mesenteric bed, the EDHF hyperpolarization[100,101] and relaxation[100-102] were significantly diminished compared with responses from control animals. EDHF-induced relaxations were also reduced *in vivo* in the renal circulation, with the most severe deficit occurring in the smallest arterioles[103]. The EDHF relaxation was also impaired in the renal artery of obese Zucker rats, which is an animal model of insulin resistance and Type II diabetes[104]. However, in a mouse model of Type II diabetes, the *db/db* -/-, the EDHF relaxation of first order mesenteric arteries was not diminished[105], indicating that EDHF is not impaired in all models of diabetes. The

mechanisms underlying disease-associated impairment of EDHF-attributed hyperpolarization and relaxation are far from clear and require further studies to determine whether the dysfunction arises in the smooth muscle cells, and/or the endothelial cells, and/or myoendothelial communication[106]. This knowledge could provide the basis of novel therapeutic interventions in the amelioration or prevention of vascular complications of these diseases.

Conclusions

In many vessels, abolition of EDHF-attributed relaxation and/or hyperpolarization by apamin combined with ChTx, but not IbTx, or with a TRAM compound, implicate SK_{Ca} and IK_{Ca} as the ion channels carrying the current which underlies the EDHF hyperpolarization. Biophysical properties of the EDHF current, obtained from voltage-clamp results, strongly support the involvement of these channels and exclude the involvement of other ionic mechanisms such as K_{IR} channels and the Na^+/K^+ ATPase, at least in submucosal arterioles. In some vessels, EDHF is attributed to a product of the cytochrome P450 pathway and to involve the activation of BK_{Ca} channels. However, the poor selectivity of many blockers of cytochrome P450 pathways and differences in the actions of various agonists applied to stimulate the endothelial cells, means that further studies are required to better understand the role of the cytochrome P450 pathway in the EDHF response.

IK_{Ca} and SK_{Ca} channels occur in abundance on endothelial cells but not on smooth muscle cells and endothelial cells respond to agonists with EDHF-like hyperpolarization. Furthermore, there is strong myoendothelial electrical coupling in vessels with EDHF responses, but not in vessels without EDHF, although the range of vessels that have been tested is limited. Together, these observations suggest that EDHF likely involves the activation of K_{Ca} channels in the endothelial cells, and that the EDHF hyperpolarization of smooth muscle involves the spread of hyperpolarizing current from the endothelium via myoendothelial gap junctions. Some variations between vascular beds and species in the relative effectiveness of apamin, ChTx and IbTx is likely to reflect differences in the relative densities of the K_{Ca} channels. BK_{Ca} channels may be important in some vessels, while IK_{Ca} and SK_{Ca} channels are more important in many other vascular beds. These endothelial channels make an important contribution to vascular tone *in vivo*, and impairment of their effectiveness contributes to endothelial dysfunction in a range of diseases, thus raising the mechanisms underlying EDHF as potential therapeutic targets.

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