Factors, fiction and endothelium-derived hyperpolarizing factor - EDH(F)

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Summary

1. The principal mediators of vascular tone are neural, endothelial and physical stimuli that result in the initiation of dilator and constrictor responses to facilitate the control of blood pressure. Two primary vasodilatory stimuli produced by the endothelium are nitric oxide (NO) and prostaglandins. An additional endothelium dependent vasodilatory mechanism is characterized as the hyperpolarization mediated relaxation that remains after the inhibition of the synthesis of NO and prostaglandins. This mechanism is due to the action of a so-called endothelium-derived hyperpolarizing factor (EDHF) and is dependent on either the release of diffusible factor(s) and/or to a direct contact-mediated mechanism.

2. Most evidence supports the concept that 'EDHF' activity is dependent on contact-mediated mechanisms. This involves the transfer of an endothelium-derived electrical current, as an endothelium-derived hyperpolarization (EDH), through direct heterocellular coupling of endothelial cells (ECs) and smooth muscle cells (SMCs) via myoendothelial gap junctions (MEGJs). However, there is a lack of consensus with regard to the nature and mechanism of action of EDHF/EDH (EDH(F)), which has been shown to vary within and between vascular beds, as well as among species, strains, sex and during development, ageing and disease.

3. In addition to actual heterogeneity in EDH(F), further heterogeneity has resulted from the less than optimal design, analysis and interpretation of data in some key papers in the EDHF literature; with such views being perpetuated in the subsequent literature.

4. The focus of this brief review is to examine what factors are proposed as EDH(F), and highlight the correlative structural and functional studies from our laboratory that demonstrate an integral role for MEGJs in the conduction of EDH which account for the heterogeneity in EDH(F); whilst incorporating the reported diffusible mechanisms in the regulation of this activity. Furthermore, in addition to the reported heterogeneity in the nature and mechanism of action of EDH(F), the contribution of experimental design and technique to this heterogeneity will be examined.

What is EDH(F)?

The aim of this brief review is to provide a critical overview of the EDH(F) field, with a focus on the role of gap junctions in the EDH(F) phenomenon. More extensive reviews on EDHF are provided by McGuire *et al*.,[1] Campbell and Gauthier,[2] Ding and Triggle,[3] and Griffith.[4]

Briefly, the arterial endothelium produces three vasodilatory factors; NO, prostaglandins and EDH(F). Classically, EDH(F) is the hyperpolarization and associated relaxation remaining after the inhibition of the synthesis of NO synthase (and thus NO) and prostaglandins. The two primary mechanisms that can account for EDH(F) activity rely on either diffusible- and/or contact-mediated mechanisms. Those that are dependent on the release of a diffusible substance, for which there is yet to be unequivocal evidence, are due to EDHF. Those that are dependent on the direct contact of ECs and SMCs via MEGJs are due to the transfer of an electrical current, as an EDH.[4-9] In both cases, the net result is the hyperpolarization of the adjacent smooth muscle with subsequent vessel dilation. For clarity the term EDH(F) will be used here to refer to both a diffusible or contact-mediated mechanism.

Regardless of whether a diffusible- or contact-mediated mechanism is involved in EDH(F) activity, it is accepted that its action is dependent on the release of intracellular calcium and the activation of a specific pattern of potassium channels. The activation of receptors and/or application of physical stimuli such as shear stress results in a rise in intracellular EC calcium.[1,4,10] Subsequently, this results in the activation of small (S) and intermediate (I) conductance calcium activated potassium channels (K_{Ca}) located on ECs, and in some cases the activation of EC or SMC large (B) K_{Ca}.[1] This channel activation results in the generation of an EDH or the release of an EDHF, which is subsequently transmitted to the adjacent SMC layer either via MEGJs or by diffusion.[1,2] Indeed, it is agreed that EDH(F) activity is blocked by the application of K_{Ca} antagonists, such as apamin (SK_{Ca} antagonist) and charybdotoxin (non-selective IK_{Ca} and BK_{Ca} antagonist, with additional effects at voltage-dependent potassium channels[3]) in combination,[1,2] or apamin and TRAM-34 (IK_{Ca} antagonist) in combination,[4,11,12] in the case of SK_{Ca} and IK_{Ca} dependent responses, or by iberiotoxin in the case of BK_{Ca} dependent responses.[2]

The nature and mechanism of EDH(F) apparently

varies within and between vascular beds and amongst species, strains, sex and during development, ageing and disease,[1-3] as well as with variable experimental conditions and between laboratories.[4] A proposal for unifying the role of EDH(F) and heterocellular coupling has recently been put forward by Griffith[4] This scheme incorporates many of the proposed EDH(F)s, and questions others, for which there is debatable evidence.

Diffusible factors

Contact-mediated mechanisms represent the simplest explanation of EDH(F) activity, as a purely electrical event. However, the release of diffusible factors/s from the endothelium, at a concentration sufficient to change that of the internal elastic lamina and the local environment surrounding the innermost layer of SMCs, has also been proposed to account for EDH(F) activity. This substance then putatively effects the activation of SMC receptors and ion channels, to initiate smooth muscle hyperpolarization and relaxation.[1-4]

Diffusible factors proposed as an EDHF include K^+ ions, epoxyeicosatrienoic acids (EETs), H_2O_2,[1,2] and C-type natriuretic peptide (CNP[13]). N^{ω}-nitro-L-arginine methyl ester (L-NAME) insensitive nitric oxide has also been suggested to account for EDHF activity.[14,15] In addition, S-nitrosothiols have been suggested to contribute to EDHF activity,[16] although the evidence for the endothelial dependence of this response requires further investigation.

Potassium ions

Several studies have supported the proposal that K^+ ions are an EDHF in some vessels (for references see [1,3,4,17]). Indeed, since the original proposition that K^+ ions were an EDHF, this hypothesis has received much attention. Basically, this scheme involves the activation of EC K_{Ca} and the subsequent EC efflux of K^+ from these channels. The resultant potassium 'cloud'[17] then reportedly diffuses across the internal elastic lamina to act as an EDHF by evoking smooth muscle hyperpolarization and relaxation, via the activation of smooth muscle Na^+/K^+ATPase and inwardly rectifying potassium channels;[17] key channels for the modulation of ionic mechanisms that are reportedly sensitive to the application of ouabain and barium, respectively. Antagonism of the EDHF response by these blockers is used as defining evidence for K^+ as an EDHF. In its current form this mechanism is referred to as the 'potassium cloud hypothesis'.[17]

A complication to this hypothesis is the efflux of K^+ from SMCs that arises as a result of depolarization, which would thus contribute to the basal level of K^+ surrounding vascular cells, and will thus suppress the K^+/EDHF effect. At a simplistic level the term 'potassium cloud' is misleading, in that it implies the presence of a global cloud of potassium surrounding the vascular cells, when in fact any physiologically relevant change in the K^+ concentration will be transient and localized. Indeed, a more plausible scenario is that the K^+ flux acts at restricted localized sites (microdomains), as has been described in SMCs and other cell types.[18]

Interestingly, the most recent version of the 'potassium cloud hypothesis' includes a role for MEGJs in the action of K^+ as EDHF.[17] However, once a role for MEGJs is included in this mechanism, a role for K^+ as a diffusible EDHF may be redundant, since the EDHF phenomenon can be simply explained through the action of EDH. As alluded to above, a potential scenario where the diffusion of K^+ may play a role in the EDHF activity could arise if there is a close spatial relationship between MEGJs and K_{Ca} distribution (as well as perhaps with sites of calcium extrusion), in the form of microdomains, where highly localized changes in K^+ concentrations could play a role in the coordination and modulation of heterocellular-EDH(F) signaling (Garland and Sandow, personal communication). Whilst evidence for similar functional microdomains in SMCs and other cell types is well documented,[18] it is interesting to speculate that this scenario may be the case in ECs of resistance vessels such as the mesenteric bed of the rat where functional studies have suggested this to occur.[19] Further anatomical support for the existence of microdomains in ECs is not currently available in resistance vessels, and thus a role for a K^+ in this scenario is speculative.

Epoxyeicosatrienoic acids (EETs)

There is evidence of a role for EETs in EDH(F) activity in some vascular beds.[1,2] EETs are cytochrome P450 expoxygenase metabolites of phospholipase dependent arachidonic acid production, which putatively activate smooth muscle BK_{Ca}[20] to result in hyperpolarization and arterial relaxation in cerebral, coronary and renal arteries of several species.[1,2] Indeed, although there is evidence that EETs play an integral role in EDH(F) activity in some vascular beds, EETs are not a universal EDH(F), in that in many vascular beds, EDH(F) activity is not sensitive to the application of iberiotoxin, a BK_{Ca} antagonist.[4] Furthermore, it is not clear if EETs activity is related to their participation in the facilitation of autocrine pathways that generate hyperpolarization via mechanisms that are indistinct from alternative agonist-induced pathways that result in an analogous activation of an EDH(F) type response.[4]

Hydrogen peroxide

In human and mouse mesenteric and porcine coronary arteries, H_2O_2 has been proposed to act as an EDHF.[21-24] However, a primary problem with these studies is that the appropriate time and concentration controls for catalase, as a H_2O_2 antagonist, were not undertaken and indeed the proposal that H_2O_2 is an EDHF in these vascular beds is not consistent with several other studies undertaken in the same vascular beds (see below). Beny and von der Weid,[25] for example, have shown that EDHF and H_2O_2 are distinct factors in porcine coronary arteries, whilst Pomposiello *et al*.[26] demonstrate that catalase, an enzyme inhibitor H_2O_2 of activity, has no effect in porcine coronary vessels; although at 300U/ml it did abolish the endothelium

independent relaxation to exogenously generated H_2O_2 after 45min incubation. Catalase has been shown to have no effect on EDHF in the bovine ciliary, rat saphenous and mesenteric and human radial and subcutaneous arteries.[9,27-30]. In this light, several studies have shown that H_2O_2 can cause a vasoconstriction (see [31,32], for example) which can be attenuated by a 20min incubation in 100U/ml catalase.[33] Furthermore, in a membrane potential independent manner, reactive oxygen species such as H_2O_2 have been reported to variably activate SMC K_{Ca}, ATP-sensitive potassium channels, Na^+/K^+ATPase and modulate the sensitivity of the contractile apparatus to calcium,[4,10] thus playing additional roles unrelated to EDHF, but complicating any speculative role for H_2O_2 in EDHF activity. Indeed, in contrast to the original proposition that H_2O_2 was an EDHF in mouse mesenteric vessels Ellis *et al.*[34] provide evidence that H_2O_2 is not an EDHF in these vessels. Indeed, Ellis *et al.*[34] found that an inhibitory effect of catalase does not provide definitive evidence that H_2O_2 is critical to a given vascular response.[10]

In any event, the physiological relevance of H_2O_2 as an EDHF is simply questioned based on the observation that the concentration of H_2O_2 produced in response to endothelial stimulation (10-60nM[35]; see [4]) is substantially less than the 3μM to 100μM of H_2O_2 required to elicit a 30 to 90% relaxation in human mesenteric vessels[22] or the 0.1mM and 1mM of H_2O_2 required to elicit a 60 and 100% relaxation in porcine coronary arteries.[25] In addition, concentration dependent effects of H_2O_2 are critical to the question of whether physiological or pathophysiological effects are observed, since H_2O_2 can mediate vascular cell proliferation, apoptosis, hyperplasia, cell adhesion and migration, as well as having effects on arterial tone.[10] Indeed, predominant evidence supports the proposition that H_2O_2 is not involved in the hyperpolarization dependent EDHF response and that it is not an EDHF.[10,36]

C-type natriuretic peptide (CNP)

C-type natriuretic peptide has been proposed to act as an EDHF[13] and indeed the data presented in Chauhan *et al.*[13] are consistent with the activation of the CNP receptor C subtype playing a role in the EDH(F) phenomenon. However, in the same mesenteric vessels as examined in Sprague-Dawley rats by Chauhan *et al.*,[13] but in the mature Wistar rat, Sandow *et al.*[37] demonstrate that heterocellular coupling of ECs and SMCs accounts for EDH activity in this bed. Whilst the difference between the two studies could be related to strain variation, such a fundamental difference is unlikely and the specific reason for the discrepancy is unknown. Interestingly, in this light, the use of the non-selective gap junction antagonist glycyrrhetinic acid (GA) and its derivatives have implicated a primary role for gap junctional coupling in EDH(F) activity in this vascular bed.[38-41] Indeed, Chauhan *et al.*,[13] implicate a role for MEGJs in the proposal that CNP is EDH(F) via the use of α-GA, although at present this role is currently unknown, but is being investigated (Ahluwalia, personal communication). In any case, a role for MEGJs in the activity of CNP as EDH(F) is based on the assumption that GA is a specific antagonist for MEGJs and since no control studies for the effects of GA were undertaken in the Chauhan *et al.*[13] study, this claim is open to question. Indeed, GA and its derivatives have been shown to block homocellular and MEGJs in this vessel,[39,41] as well as having direct effects on the EC hyperpolarization to acetylcholine (ACh), via effects on phospholipase activity, and EC SK_{Ca}, IK_{Ca} and Na^+/K^+ATPase, irrespective of its putative effect at gap junctions.[6,42] A limitation of future studies examining a potential role for CNP as an EDH(F), is the lack of availability of selective antagonists for the CNP receptor-C subtype that is reported to mediate this response. Furthermore, specific limitations of the Chauhan *et al.* study[13] include; the lack of a demonstration that the CNP-mediated relaxation can occur independently of the endothelium (which would thus demonstrate CNP action at the smooth muscle) and a lack of explanation of the observations that CNP evokes ~60 to 70% relaxation, whilst EDHF evokes ~100% relaxation. Additionally, there is also a lack of explanation as to why the (non-specific) blockade of gap junctions with GA suppresses CNP activity, or what effects barium alone has on the CNP- and EDHF-mediated relaxations, or the inclusion of appropriate control data to determine if there was a basal release of CNP in these mesenteric vessels. Thus, a definitive role for CNP in EDH(F) activity remains to be elucidated.

L-NAME insensitive nitric oxide

Endogenous or basal NO activity, which is insensitive to the application of NO synthase antagonists used in the routine study of EDH(F), has been suggested to account for EDH(F) activity.[14,15] Current evidence suggests that in some vascular beds, under specific experimental conditions, this L-NAME insensitive NO may account for a *minor* degree of EDH(F) activity and one not consistently observed in studies of the same vascular bed. For example, in the Chauhan *et al.* study,[15] purporting to show that L-NAME insensitive NO accounts for a *significant* portion of EDH(F) activity, 63% of hyperpolarization and 70% of relaxation to ACh remain after the addition of the NO scavenger oxyhaemoglobin (in the presence of L-NAME and indomethacin). Furthermore, in the caudal and saphenous arteries of the rat and mesenteric artery of the mouse the NO scavengers hydroxocobalamin and carboxy-PTIO have no effect on EDH(F);[9,24,43] thus demonstrating a lack of an L-NAME insensitive NO component in these vascular beds. The contribution of endogenous NO to EDH(F) activity therefore appears variable and in many cases non-existent. Further studies are required to determine the physiological relevance of this phenomenon.

Contact-mediated mechanisms

Evidence supporting the critical role of MEGJs in EDH(F) activity comes primarily from structural and functional studies from our laboratory in Canberra and Tudor Griffith's[4,36,44,45] laboratory in Cardiff. These studies, which illustrate the simplest explanation of EDH(F)

activity, utilize the electron microscopic identification of MEGJs, electrophysiological recordings from dye identified ECs and SMCs and myography with pharmacological interventions, as well as immunohistochemical methods for identifying the connexins and ion channels involved in the EDH(F) phenomenon. These studies are consistent with the hypothesis that EDH(F) is an electrical phenomenon involving the gap junctional transfer of an EDH, from ECs to the innermost layer of intimal SMCs in the arterial wall, for the subsequent generation of an arterial relaxation.

Studies from our laboratory, which are the focus of this section of the review, have examined the role of MEGJs in EDH activity. We have found that the distribution and activity of MEGJs is correlated with the presence of EDH within and between vascular beds, during development and in disease. In the proximal and distal mesenteric arteries of the rat, for example, gap junctions play a critical role in EDH activity,[39,41] where MEGJs are prevalent.[46] In this vascular bed, in collaborative studies with Marianne Tare in Helena Parkington's laboratory in Melbourne, we showed that the presence of EDH is correlated with the presence of MEGJs, whilst in the femoral artery a lack of MEGJs is correlated with the absence of EDH.[37] A similar situation is present in the lateral saphenous artery of the juvenile rat, where MEGJs are prevalent and EDH-mediated relaxation present.[9] This is in contrast to the saphenous artery of the adult, where MEGJs were rare and EDH absent.[9] The relationship between EDH and MEGJs is somewhat more complicated in disease states, such as in hypertension. In a comparative study of the caudal artery of the hypertensive SHR and normotensive WKY rat, EDH activity was maintained, in spite of an increase in the number of SMC layers in the vessels from the hypertensive rat. This maintenance was found to be correlated with a concomitant increase in the incidence of MEGJs in the caudal artery of the hypertensive rat.[43]

The above studies demonstrate there is a direct relationship between the degree of EDH and the incidence of MEGJs. Indeed, EDH increases with an increase in the number of MEGJs per EC, whilst, conversely, it generally decreases with an increase in the number of SMC layers and vessel diameter (Figure 1). Interestingly, whilst EDH is the predominant vasodilator in smaller vessels, it is present in some larger vessels (Figure 1), such as the rabbit iliac, rat caudal and superior mesenteric arteries.[41,43,45] In the rabbit iliac artery cAMP has been proposed to enhance the spread of EDH via modulating gap junctional coupling within the multiple SMC layers, as well as at MEGJs.[47] Whilst conclusive biophysical evidence for this mechanism being relevant in larger vessels is lacking,[48] this mechanism may be of some importance for EDH activity in larger vessels.

These studies demonstrate that there is a consistent positive correlation between MEGJs and EDH activity within and between vascular beds and during development and disease. Whilst this correlation is not definitive evidence that contact-mediated mechanisms account for EDH(F) activity, to date, these data provide the most conclusive and plausible explanation for this activity.

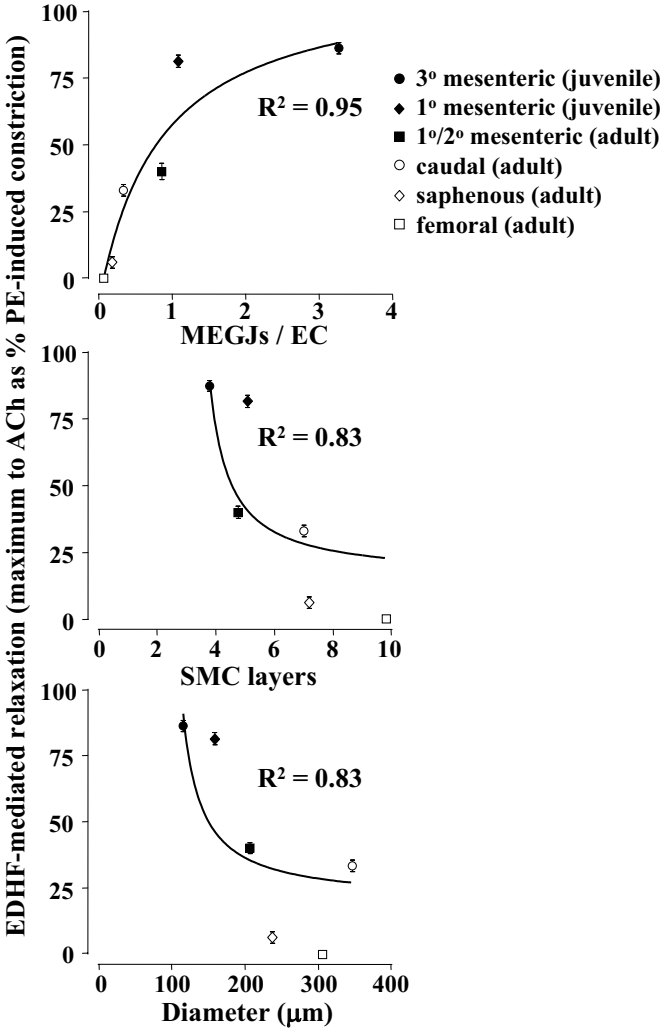

Figure 1. *Summary data demonstrating the relationship between acetylcholine (ACh)-induced EDH(F) activity and arterial morphology as the number of myoendothelial gap junctions (MEGJs) per endothelial cell (EC), per number of medial smooth muscle cell (SMC) layer and per vessel diameter. Individual data points are presented as the mean ± SEM with data being derived from earlier studies.[9,37,43,46] Data were fitted with a one phase exponential curve using Graphpad Prism. PE, phenylephrine.*

Role of diffusible factors in contact-mediated mechanisms

Direct electrical coupling is the most plausible mechanism to fully account for EDH activity. Indeed, there is increasing evidence that the diffusible factors, that act as credible EDHFs, may in fact be associated with the modulation of gap junction activity and specifically of MEGJs,[4] for the transfer of EDH, as the most plausible mechanism of their activity. These mechanisms are outlined below.

Potassium ions

The original hypothesis regarding the mechanism of action of K^+ as EDHF has been modified to include a role for MEGJs.[17] However, although K^+ are involved in mediating EDH(F) activity, once a role for such MEGJs is included, no direct role for K^+ as a diffusible EDHF is necessary for the transfer of an EDH. Indeed, in a series of experiments that repeated those in the original proposal that K^+ was EDHF, the data in the original study could not be repeated.[49] In addition, several studies have questioned the nature of K^+ as EDHF, since barium and ouabain, which are used to define the role of K^+ as an EDHF, do not universally block EDHF-mediated responses (for references see [1,3,4,17,50]). Indeed, the efficacy of ouabain as a selective Na^+/K^+ATPase antagonist has been questioned,[4,51,52] whereby it has inhibitory effects on cell coupling via modulating gap junction function.[53] Indeed, ouabain may directly attenuate the transfer of EDH by its action at gap junctions.[4,51,52] This action includes direct effects on gap junctional coupling, such as reducing connexin (Cx) expression through reduced Cx trafficking to the cell membrane, as well as modulating gap junction conductance.[52] The implication of these observations is that the attenuation of an EDH(F) response by ouabain, as with high concentrations of potassium, does not necessarily provide evidence of the EDH(F) nature of the response.[4,6,52] The demonstration that ouabain has direct effects on gap junctions, and thus on EDH(F), are essentially control studies for the earlier work that relied on the use of ouabain to show that K^+ was EDHF. Thus, based on these 'control' data[4,51,52] K^+ ions are not an EDH(F), but rather may simply be involved in the modulation of the signal transduction pathways associated with gap junction function[54,55] and thus with EDH activity.[4] Further investigation is required to elucidate any potentially specific effects of ouabain on vasomotor responses and those at gap junctions. Indeed, this point is critical for the accurate interpretation of future EDH(F) data.

Epoxyeicosatrienoic acids (EETs)

In studies of cultured ECs, EETs have been shown to modulate homocellular gap junctions,[56] thus providing a potential mechanism for a modulatory role for EETs in EDH action.[4] Griffith[4] suggests that EETs activity may be related to a complex interaction of calcium and potassium homeostasis, cAMP and arachidonic acid activity and electrotonic signaling (see Figure 3 in [4] and also [50]). Indeed, EETs have also been suggested to be modulate EC K_{Ca} activity,[57] thus providing a further mechanism for their potential role in modulating EDH, independent of acting directly as an EDHF. Further studies of the role of EETs in EDH activity in intact vessels are required to clarify these proposals.

Hydrogen peroxide

There is some evidence that H_2O_2 can effect gap junction activity and calcium homeostasis; two factors that are integral for EDH activity. Depending on the experimental conditions, studies have shown that H_2O_2 can both increase[58] and decrease[59] gap junctional coupling, and effect changes in intracellular calcium homeostasis, both in cultured cells and in intact arteries.[59-61] Although no specific evidence is currently available to support this proposal, these observations provide potential support for a mechanism to link the putative role of H_2O_2 as an EDH(F), with the MEGJ dependence of the EDH phenomenon.

C-type natriuretic peptide (CNP)

The putative action of CNP as an EDH(F)[13], may be via acting as yet another factor that facilitates electrical coupling through gap junctions; although any putative mechanism for this is unknown. Indeed, any putative action for CNP as EDH(F) cannot be directly associated with the gap junctional transfer of CNP from ECs to SMCs, since gap junctions are limited to passing substances of ≤ 1kD and CNP has a molecular weight of ~2.2kD (Ahluwalia, personal communication). Interestingly, in the Chauhan *et al.* study[13], proposing that CNP is an EDH(F), the response is sensitive to the combination of barium and ouabain, an observation that this is not a universal characteristic of EDH(F) in this, the rat mesenteric vascular bed.[49] Indeed, since ouabain is recognized as a non-specific gap junction antagonist, this result may in fact reflect a MEGJ dependence of EDH(F) in the mesenteric bed of the rat, as demonstrated by Sandow *et al*.[37]

Myoendothelial gap junctions, EDH and gap junction inhibitors

The demonstration of the dependence of EDH activity on gap junctions relies, in part, on the specific pharmacological inhibition of gap junctions. Unfortunately, there are a number of limitations regarding this methodology. The primary one of these relates to the dependence on the use of gap junction inhibitors that have not been adequately characterized in terms of their specificity and mechanism of action. Currently, there is no unequivocal evidence that the available gap junction inhibitors are specific;[62] let alone selective for gap junctions, be they heterocellular or homocellular. Indeed, unfortunately to date, few studies have examined this problem in detail and few have carried out the defining experiment of examining the effect of these agents on cell input resistance, whereby an increase in input resistance would provide key data on the gap junction antagonist effects of these agents. Of the studies that have carried out such technically demanding experiments, the data are not consistent and are incomplete; although this may in part reflect the heterogeneity in the Cx composition of vascular gap junctions.[63]

Much of the current evidence for the gap junction and specifically MEGJ dependence of EDH relies on the utilization of the licorice derivatives (the GAs and carbenoxolone; see above for an outline of non-specific actions), the Cx-mimetic peptides (Gap26,[43] Gap27,[40] Gap27;[37,43] which, based on putative selectivity, are the

current gap junction inhibitors of choice[4,9,44]) and decreasingly, with the long chain alcohols, such as heptanol. However, there is little equivocal evidence that these agents are gap junction specific and that they do not induce other non-gap junctional effects. Whilst there is well documented (and often ignored) evidence for the non-specific effects of the licorice derivatives (see above) and heptanol (for example, [64]) the Cx-mimetic peptides, have not yet been equivocally tested for specificity, nor is their mechanism of action known. In this regard, a primary issue with the use of the Cx-mimetic peptides relates to the apparent requirement to use very high concentrations and long incubation times to attenuate gap junction activity.[4] Interestingly, others report significant effects with lesser concentrations of the peptide/s and reduced incubation times.[62,65,66] Clearly, there is a pressing need for these issues to be addressed.

Why is there such a disparity of views as to the nature and mechanism of action of EDH(F)?

The conventional reason given for the disparity of views as to the nature and mechanism of action of EDH(F) is that there is heterogeneity within and between arteries, species, sex, strain and disease states.[1-4,10,17] However, a further cause of the heterogeneity relates to the less than optimal design, analysis and interpretation of data present in some key papers in the EDH(F) literature. Whilst some earlier studies can be seen as flawed with hindsight, this is not necessarily the case, since they may in fact represent significant contributions to the EDHF literature through their role in advancing the evolution of the field. Unfortunately, this is not always the case, and the perpetuation of now potentially misleading data is problematic. In any case, it is recognized that there is variation in the nature and mechanism of EDH(F) between laboratories,[4] thus questioning the relevance of the data and conclusions of some studies.

The problems of experimental technique, with regard to the design, analysis and interpretation of data that contribute to the reported heterogeneity in the nature and mechanism of action of EDH(F) in the literature include:

1. The use of *selected* agonists, antagonists and/or modulators of the investigators choice and interest, but not those which may indicate an alternative nature or mechanism of EDH(F)(for references see [1,3,4,17]). That is, for example, an investigator may be interested in EETs or gap junctions to account for EDH(F) activity, but may thus limit the investigation to the use of antagonists of the mechanism of their interest, rather than of alternative pathways. This results in a potential for a bias in favor of a particular putative EDH(F)(for references see [1,3,4,17]).

2. The lack of control data for the effects of agonists, antagonists and other modulators. For instance, in the Matoba *et al*. studies[21-23] examining the role of H_2O_2 as an EDH(F), justification should be provided as to the incubation time with catalase (2 hours or longer) as well as the high (and variable) concentration of catalase that was used.

3. The clear need for greater transparency with regard to variability in cell, vessel and species specific responses, as a result of a specific receptor and channel population, and associated signal transduction pathways (for references see [63]).

4. Making inappropriate comparative analyses between studies, including a lack of consideration of strain,[67,68] age,[9,69-71] sex,[72-75], the use of intact versus isolated tissue and tension versus pressurized myography (for references see page 15 in [63]), as well as variation in the classification of arterial branching patterns.[39,41,46] Indeed, such characteristics are often not stated in the methods section of papers and thus result in an inability to make comparative analyses between studies.

5. Lack of clarity and relevance as to the experimental protocol. For instance, under conditions of little or no vascular tone, use of buffers [such as HEPES],[77] that have non-physiological effects, the use of preconstrictor agents that adversely effect channel activity[4] such as the effect of U46619 on SK_{Ca} [78] and the effect of the GA and related compounds on a variety of cell processes, as outlined above.

6. Extrapolation of data to other vascular beds. For example, Chauhan *et al*.[13,15] examined EDH(F) in mesenteric vessels of the mature male Sprague Dawley rat, but extrapolate the data to be applicable to the vasculature as a whole. Whilst several studies have made such claims (for references see [1,3,4]), this contention merely confuses the field, as there is no evidence to justify this point of view.

Conclusions

The nature and mechanism of action of EDH(F) can apparently differ along and between vascular beds, between species, strains, sex and during development, ageing and disease. This heterogeneity can be explained through the action of heterocellular coupling. Indeed, contact-mediated mechanisms represent the simplest explanation of EDH(F) activity and involve the transfer of an endothelium-derived electrical signal to the smooth muscle via MEGJs, as EDH. Of the putative diffusion-mediated mechanisms, K^+ ions have received much attention in the literature and whilst they might not be an EDHF, they are involved in the signal transduction pathways associated with the generation of the EDH and they may be involved in the modulation of gap junction activity. In a similar manner, there is good evidence of a role for EETs in EDH(F) activity in some vascular beds, although this role may be confined to a modulatory role of homo- and heterocellular coupling, as well as modulating the K_{Ca} component of the EDH mechanism. The role of CNP as an EDH(F) is yet to be clarified, but may also be related to the modulation of EDH

activity. Predominant evidence supports the proposition that H_2O_2 is not an EDH(F), although again, its activity may also be related to the modulation of gap junction function, and thus of EDH. L-NAME insensitive NO may account for a degree of EDH(F) activity in some vascular beds, but the extent of this is limited to only a minor part of such activity. Whilst the nature and mechanism of action of EDH(F) is in part be due to actual heterogeneity, it is also unfortunately due to a lack of consistent and sound scientific methodology.

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References

1. McGuire JJ, Ding H, Triggle CR. Endothelium-derived relaxing factors: a focus on endothelium-derived hyperpolarizing factor(s). *Can. J. Physiol Pharmacol.* 2001; **79**: 443-470.
2. Campbell WB, Gauthier KM. What is new in endothelium-derived hyperpolarizing factors? *Curr. Opin. Nephrol. Hyperten Res.* 2002; **11**: 177-183.
3. Ding H, Triggle CR. Contribution of EDHF and the role of potassium channels in the regulation of vascular tone. *Drug Dev. Res.* 2003; **58**: 81-89.
4. Griffith TM. Endothelium-dependent smooth muscle hyperpolarization: do gap junctions provide a unifying hypothesis? *Brit. J. Pharmacol.* 2004; **141**: 881-903.
5. Yamamoto Y, Imaeda K, Suzuki H. Endothelium-dependent hyperpolarization and intercellular electrical coupling in guinea-pig mesenteric arterioles. *J. Physiol.* 1999; **514**: 505-513.
6. Coleman HA, Tare M, Parkington HC. K^+ currents underlying the action of endothelium-derived hyperpolarizing factor in guinea-pig and human blood vessels. *J. Physiol.* 2001; **531**: 359-373.
7. Coleman HA, Tare M, Parkington HC. EDHF is not K^+ but may be due to spread of current from the endothelium in guinea pig arterioles. *Am. J. Physiol.* 2001; **280**: H2478-H2483.
8. Coleman HA, Tare M, Parkington HC. Myoendothelial electrical coupling in arteries and arterioles and its implications for endothelium-derived hyperpolarizing factor. *Clin. Exp. Pharmacol. Physiol.* 2002; **29**: 630-637.
9. Sandow SL, Goto K, Rummery N, Hill CE. Developmental dependence of EDHF on myoendothelial gap junctions in the saphenous artery of the rat. *J. Physiol.* 2004; **556**: 875-886.
10. Ellis A, Triggle CR. Endothelium-dependent reactive oxygen species: their relationship to endothelium-dependent hyperpolarization and the regulation of vascular tone. *Can. J. Physiol. Pharmacol.* 2003; **81**: 1013-1028.
11. Crane GJ, Walker SD, Dora KA, Garland CJ. Evidence for a differential cellular distribution of inward rectifier K channels in the rat isolated mesenteric artery. *J. Vasc. Res.* 2003; **40**: 159-168.
12. Eichler I, Wibawa J, Grgic I, Knorr A, Brakemeier S, Pries AR, Hoyer J, Kohler R. Selective blockade of endothelial Ca(2+)-activated small- and intermediate-conductance K(+)-channels suppresses EDHF-mediated vasodilation. *Br. J. Pharmacol.* 2003; **138**: 594-601.
13. Chauhan SD, Nilsson H, Ahluwalia A, Hobbs AJ. Release of C-type natriuretic peptide accounts for the biological activity of endothelium-derived hyperpolarizing factor. *Proc. Natl. Acad. Sci.* 2003; **100**: 1426-1431.
14. Cohen RA, Plane F, Najibi S, Huk I, Malinski T, Garland CJ. Nitric oxide is the mediator of both endothelium-dependent relaxation and hyperpolarization of the rabbit carotid artery. *Proc. Natl. Acad. Sci.* 1997; **94**: 4193-4198.
15. Chauhan S, Rahman A, Nilsson H, Clapp L, MacAllister R, Ahluwalia A. NO contributes to EDHF-like responses in rat small arteries: a role for NO stores. *Cardiovasc. Res.* 2003; **57**: 207-216.
16. Batenburg WW, Popp R, Fleming I, de Vries R, Garrelds IM, Saxena PR, Danser AH. Bradykinin-induced relaxation of coronary microarteries: S-nitrosothiols as EDHF? *Brit. J. Pharmacol.* 2004; **In Press.**
17. Busse R, Edwards G, Feletou M, Fleming I, Vanhoutte PM, Weston A. EDHF: bringing the concepts together. *Trends Pharmacol. Sci.* 2002; **23**: 374-380.
18. Poburko D, Kuo KH, Dai J, Lee CH, van Breemen C. Organellar junctions promote targeted Ca^{2+} signalling in smooth muscle: why two mechanisms are betterthan one. *Trends Pharmacol. Sci.* 2004; **25**: 8-15.
19. Crane GJ, Gallagher NT, Dora KA, Garland CJ. Small and intermediate calcium-dependent K+ channels provide different facets of endothelium-dependent hyperpolarization in rat mesenteric artery. *J. Physiol.* 2003; **553**: 183-189.
20. Archer SL, Gragasin FS, Wu X, Wang S, McMurtry S, Kim DH, Platonov M, Koshal A, Hashimoto K, Campbell WB, Falck JR, Michelakis ED. Endothelium-derived hyperpolarizing factor in human internal mammary artery is 11,12-epoxyeicosatrienoic acid and causes relaxation by activating smooth muscle BK(Ca) channels. *Circulation.* 2003; **107**: 769-776.
21. Matoba T, Shimokawa H, Nakashima M, Hirakawa Y, Mukai Y, Hirano K, Kanaide H, Takeshita A. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. *J. Clin. Invest.* 2000; **106**: 1521-1530.
22. Matoba T, Shimokawa H, Kubota H, Morikawa K, Fujiki T, Kunihiro I, Mukai Y, Hirakawa Y,

Takeshita A. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in human mesenteric arteries. *Biochem. Biophys. Res. Commun.* 2002; **290**: 909-913.

23. Matoba T, Shimokawa H, Morikawa K, Kubota H, Kunihiro I, Urakami-Harasawa L, Mukai Y, Hirakawa Y, Akaike T, Takeshita A. Electron spin resonance detection of hydrogen peroxide as an endothelium-derived hyperpolarizing factor in porcine coronary microvessels. *Arterioscler. Thromb. Vasc. Biol.* 2003; **23**: 1224-1230.
24. Morikawa K, Shimokawa H, Matoba T, Kubota H, Akaike T, Talukder MAH, Fujiki T, Maeda H, Takahashi S, Takeshita A. Pivotal role of Cu,Zn-superoxide dismutase in endothelium-dependent hyperpolarization. *J. Clin. Invest.* 2003; **112**: 1871-1879.
25. Beny JL, der Weid PY. Hydrogen peroxide: an endogenous smooth muscle cell hyperpolarizing factor. *Biochem. Biophys. Res. Commun.* 1991; **176**: 378-384.
26. Pomposiello S, Rhaleb NE, Alva M, Carretero OA. Reactive oxygen species: Role in the relaxation induced by bradykinin or arachidonic acid via EDHF in isolated porcine coronary arteries. *J. Cardiovasc. Pharmacol.* 1999; **34**: 567-574.
27. Hamilton CA, McPhaden AR, Berg G, Pathi V, Dominiczak AF. Is hydrogen peroxide an EDHF in human radial arteries? *Am. J. Physiol.* 2001; **280**: H2451-H2455.
28. McNeish AJ, Wilson WS, Martin W. Ascorbate blocks endothelium-derived hyperpolarizing factor (EDHF)-mediated vasodilatation in the bovine ciliary vascular bed and rat mesentery. *Br. J. Pharmacol.* 2002; **135**: 1801-1809.
29. Kawabata A, Kubo S, Nakaya Y, Ishiki T, Kuroda R, Sekiguchi F, Kawao N, Nishikawa H. Distinct roles for protease-activated receptors 1 and 2 in vasomotor modulation in rat superior mesenteric artery. *Cardiovasc. Res.* 2004; **61**: 683-692.
30. Luksha L, Nisell H, Kublickiene K. The mechanism of EDHF-mediated responses in subcutaneous small arteries from healthy pregnant women. *Am. J. Physiol.* 2004; **In Press**.
31. Jin N, Packer CS, Rhoades RA. Reactive oxygen-mediated contraction in pulmonary arterial smooth muscle: cellular mechanisms. *Can. J. Physiol. Pharmacol.* 1998; **69**: 383-388.
32. Gao YJ, Lee RM. Hydrogen peroxide induces a greater contraction in mesenteric arteries of spontaneously hypertensive rats through thromboxane A(2) production. *Br. J. Pharmacol.* 2001; **134**: 1639-1646.
33. Ulker S, McMaster D, McKeown PP, Bayraktutan U. Impaired activities of antioxidant enzymes elicit endothelial dysfunction in spontaneous hypertensive rats despite enhanced vascular nitric oxide generation. *Cardiovasc. Res.* 2003; **59**: 488-500.
34. Ellis A, Pannirselvam M, Anderson TJ, Triggle CR. Catalase has negligible inhibitory effects on endothelium-dependent relaxations in mouse isolated aorta and small mesenteric artery. *Br. J. Pharmacol.* 2003; **140**: 1193-1200.
35. Cosentino F, Patton S, d'Uscio LV, Werner ER, Werner-Felmayer G, Moreau P, Malinski T, Luscher TF. Tetrahydrobiopterin alters superoxide and nitric oxide release in prehypertensive rats. *J. Clin. Invest.* 1998; **101**: 1530-1537.
36. Chaytor AT, Edwards DH, Bakker LM, Griffith TM. Distinct hyperpolarizing and relaxant roles for gap junctions and endothelium-derived H_2O_2 in NO-independent relaxations of rabbit arteries. *Proc. Natl. Acad. Sci.* 2003; **100**: 15212-15217.
37. Sandow SL, Tare M, Coleman HA, Hill CE, Parkington HC. Involvement of myoendothelial gap junctions in the actions of endothelium-derived hyperpolarizing factor. *Circ. Res.* 2002; **90**: 1108-1113.
38. Edwards G, Feletou M, Gardener MJ, Thollon C, Vanhoutte PM, Weston AH. Role of gap junctions in the responses to EDHF in rat and guinea-pig small arteries. *Br. J. Pharmacol.* 1999; **128**: 1788-1794.
39. Hill CE, Hickey H, Sandow SL. Role of gap junctions in acetylcholine-induced vasodilation of proximal and distal arteries of the rat mesentery. *J. Auton. Nerv. Syst.* 2000; **81**: 122-127.
40. Doughty JM, Boyle JP, Langton PD. Blockade of chloride channels reveals relaxations of rat small mesenteric arteries to raised potassium. *Br. J. Pharmacol.* 2001; **132**: 293-301.
41. Goto K, Fujii K, Kansui Y, Abe I, Iida M. Critical role of gap junctions in endothelium-dependent hyperpolarization in rat mesenteric arteries. *Clin. Exp. Pharmacol. Physiol.* 2002; **29**: 595-602.
42. Tare M, Coleman HA, Parkington HC. Glycyrrhetinic derivatives inhibit hyperpolarization in endothelial cells of guinea pig and rat arteries. *Am. J. Physiol.* 2002; **282**: H335-H341.
43. Sandow SL, Bramich NJ, Bandi HP, Rummery N, Hill CE. Structure, function and EDHF in the caudal artery of the SHR and WKY Rat. *Arterioscler. Thromb. Vasc. Biol.* 2003; **23**: 822-828.
44. Chaytor AT, Martin PE, Edwards DH, Griffith TM. Gap junctional communication underpins EDHF-type relaxations evoked by ACh in the rat hepatic artery. *Am. J. Physiol.* 2001; **280**: H2441-H2450.
45. Chaytor AT, Taylor HJ, Griffith TM. Gap junction-dependent and -independent EDHF-type relaxations may involve smooth muscle cAMP accumulation. *Am. J. Physiol.* 2002; **282**: H1548-H1555.
46. Sandow SL, Hill CE. The incidence of myoendothelial gap junctions in the proximal and distal mesenteric arteries of the rat is suggestive of a role in EDHF-mediated responses. *Circ. Res.* 2000; **86**: 341-346.
47. Griffith TM, Chaytor AT, Taylor HJ, Giddings BD, Edwards DH. cAMP facilitates EDHF-type relaxations in conduit arteries by enhancing electrotonic conduction via gap junctions. *Proc. Natl. Acad. Sci.* 2002; **99**: 6392-6397.

48. Diep HK, Vigmond EK, Welsh DG. Modelling of electrical communication in resistance arteries. *FASEB J.* 2004; **18**: A4236.
49. Vanheel B, Van de Voorde J: Effects of barium, ouabain and K^+ on the resting membrane potential and endothelium-dependent responses in rat arteries; In: Vanhoutte PM (ed): EDHF 2000. London, Taylor and Francis, 2001, pp 146-155.
50. Dhein S. Pharmacology of gap junctions in the cardiovascular system. *Cardiovasc. Res.* 2004; **62**: 287-298.
51. Harris D, Martin PE, Evans WH, Kendall DA, Griffith TM, Randall MD. Role of gap junctions in endothelium-derived hyperpolarizing factor responses and mechanisms of K(+)-relaxation. *Eur. J. Pharmacol.* 2000; **402**: 119-128.
52. Martin PE, Hill NS, Kristensen B, Errington RJ, Griffith TM. Ouabain exerts biphasic effects on connexin functionality and expression in vascular smooth muscle cells. *Br. J. Pharmacol.* 2003; **140**: 1261-1271.
53. Ledbetter ML, Gatto CL. Concentrations of ouabain that prevent intercellular communication do not affect free calcium levels in cultured fibroblasts. *Cell Biochem. Funct.* 2003; **21**: 363-370.
54. Giaume C, Venance L: Characterization and regulation of gap junction channels in cultured astrocytes; In: Spray DC, Dermietzel R (eds): Gap junctions in the nervous system. Austin, TX, Landes, 1996, pp 136-157.
55. Pina-Benabou MH, Srinivas M, Spray DC, Scemes E. Calmodulin kinase pathway mediates the K+-induced increase in Gap junctional communication between mouse spinal cord astrocytes. *J. Neurosci.* 2001; **21**: 6635-6643.
56. Popp R, Brandes RP, Ott G, Busse R, Fleming I. Dynamic modulation of interendothelial gap junctional communication by 11,12-epoxyeicosatrienoic acid. *Circ. Res.* 2002; **90**: 800-806.
57. Baron A, Frieden M, Beny JL. Epoxyeicosatrienoic acids activate a high-conductance, Ca(2+)-dependent K + channel on pig coronary artery endothelial cells. *J. Physiol.* 1997; **504**: 537-543.
58. Rouach N, Calvo CF, Duquennoy H, Glowinski J, Giaume C. Hydrogen peroxide increases gap junctional communication and induces astrocyte toxicity: Regulation by brain macrophages. *Glia.* 2004; **45**: 28-38.
59. Todt I, Ngezahayo A, Ernst A, Kolb HA. Hydrogen peroxide inhibits gap junctional coupling and modulates intracellular free calcium in cochlear Hensen cells. *J. Membr. Biol.* 2001; **181**: 107-114.
60. Blanc EM, Bruce-Keller AJ, Mattson MP. Astrocytic gap junctional communication decreases neuronal vulnerability to oxidative stress-induced disruption of Ca^{2+} homeostasis and cell death. *J. Neurochem.* 1998; **70**: 958-970.
61. Touyz RM. Activated oxygen metabolites: do they really play a role in angiotensin II-regulated tone? *J. Hypertens.* 2003; **21**: 2235-2238.
62. Spray DC, Rozental R, Srinivas M. Prospects for rational development of pharmacological gap junction channel blockers. *Curr. Drug Targets.* 2002; **3**: 455-464.
63. Hill CE, Phillips JK, Sandow SL. Heterogeneous control of blood flow amongst different vascular beds. *Med. Res. Rev.* 2001; **21**: 1-60.
64. Hashitani H, Suzuki H. K+ channels which contribute to the acetylcholine-induced hyperpolarization in smooth muscle of the guinea-pig submucosal arteriole. *J. Physiol.* 1997; **501**: 505-513.
65. Dahl G, Nonner W, Werner R. Attempts to define functional domains of gap junction proteins with synthetic peptides. *Biophys. J.* 1994; **67**: 1816-1822.
66. Hill CE, Haddock RE, Brackenbury T. Role of the endothelium and gap junctions in cerebral vasomotion. *FASEB J.* 2004; **18**: A3631.
67. Kurtz TW, Morris RC, Jr. Biological variability in Wistar-Kyoto rats. Implications for research with the spontaneously hypertensive rat. *Hypertension.* 1987; **10**: 127-131.
68. Louis WJ, Howes LG. Genealogy of the spontaneously hypertensive rat and Wistar-Kyoto rat strains: implications for studies of inherited hypertension. *J. Cardiovasc. Pharmacol.* 1990; **16**: S1-S5.
69. Fujii K, Ohmori S, Tominaga M, Abe I, Takata Y, Ohya Y, Kobayashi K, Fujishima M. Age-related changes in endothelium-dependent hyperpolarization in the rat mesenteric artery. *Am. J. Physiol.* 1993; **265**: H509-H516.
70. Goto K, Fujii K, Onaka U, Abe I, Fujishima M. Angiotensin-converting enzyme inhibitor prevents age-related endothelial dysfunction. *Hypertension.* 2000; **36**: 581-587.
71. Goto K, Fujii K, Kansui Y, Iida M. Changes in EDHF in hypertension and ageing: response to chronic treatment with renin-angiotensin system inhibitors. *Clin. Exp. Pharmacol. Physiol.* 2004.
72. Huang A, Sun D, Koller A, Kaley G. Gender difference in flow-induced dilation and regulation of shear stress: role of estrogen and nitric oxide. *Am. J. Physiol.* 1998; **275**: R1571-R1577.
73. Golding EM, Kepler TE. Role of estrogen in modulating EDHF-mediated dilations in the female rat middle cerebral artery. *Am. J. Physiol.* 2001; **280**: H2417-H2423.
74. Xu HL, Santizo RA, Baughman VL, Pelligrino DA. ADP-induced pial arteriolar dilation in ovariectomized rats involves gap junctional communication. *Am. J. Physiol.* 2002; **283**: H1082-H1091.
75. Huang A, Sun D, Wu Z, Yan C, Carroll MA, Jiang H, Falck JR, Kaley G. Estrogen elicits cytochrome P450-mediated flow-induced dilation of arterioles in NO deficiency. Role of PI3K-Akt phosphorylation in genomic regulation. *Circ. Res.* 2004; **94**: 245-252.
76. Richards GR, Weston AH, Burnham MP, Feletou M,

Vanhoutte PM, Edwards G. Suppression of K(+)-induced hyperpolarization by phenylephrine in rat mesenteric artery: relevance to studies of endothelium-derived hyperpolarizing factor. *Br. J. Pharmacol.* 2001; **134**: 1-5.

77. Edwards G, Feletou M, Gardener MJ, Glen CD, Richards GR, Vanhoutte PM, Weston AH. Further investigations into the endothelium-dependent hyperpolarizing effects of bradykinin and substance P in porcine coronary artery. *Br. J. Pharmacol.* 2001; **133**: 1145-1153.
78. Crane GJ, Garland CJ. Thromboxane receptor stimulation associated with loss of SKCa activity and reduced EDHF responses in the rat isolated mesenteric artery. *Brit. J. Pharmacol.* 2004; **142**: 43-50.

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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

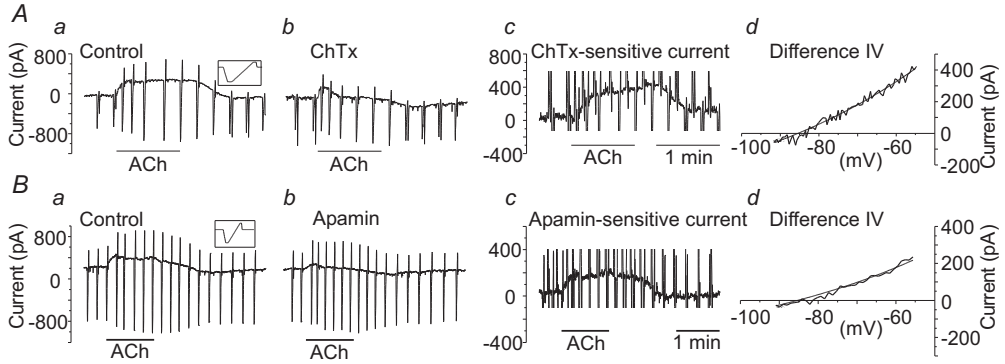

Figure 1. Components of EDHF current recorded from segments of guinea-pig submucosal arterioles.
Aa, Ba, ACh (1 μM) evoked an outward, EDHF current with the membrane clamped at −63 mV. Periodic transients are responses to voltage ramps (insets). Ab, ChTx (30 nM) and Bb, apamin (0.5 μM) reduced the EDHF 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

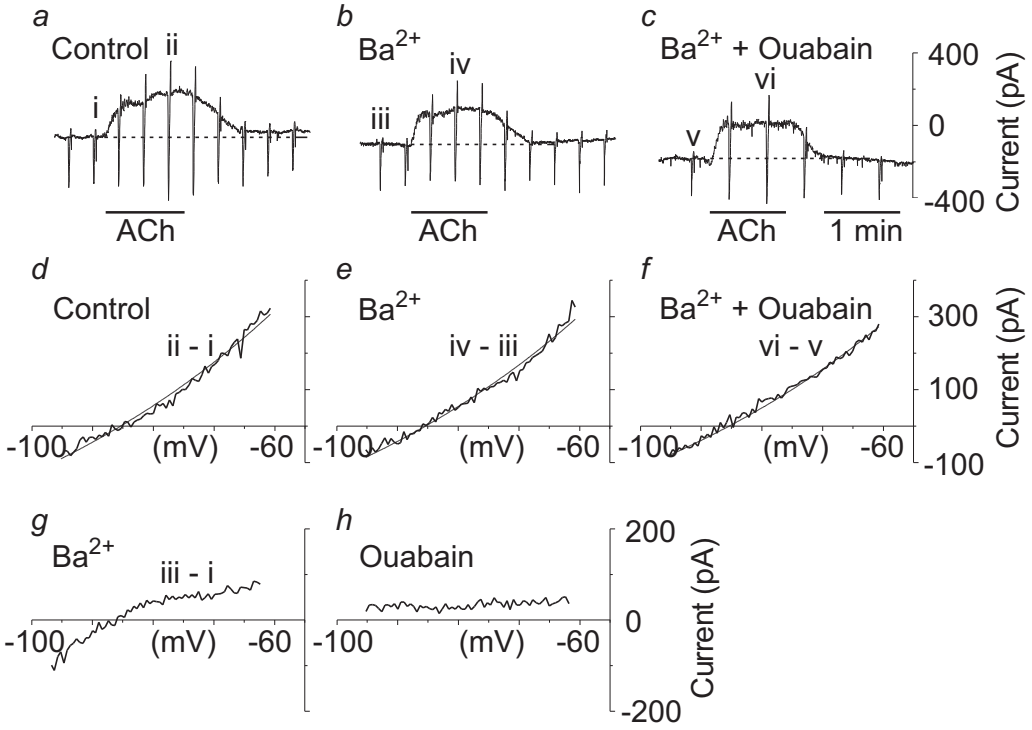

Figure 2. Contribution of K_{IR} and Na^+/K^+ATPase to arteriole currents
a, ACh (1 μM) evoked an outward, EDHF current. b, the EDHF current was not reduced by Ba^{2+} (30 μM), or c, by the addition of ouabain (200 μM) in the continuing presence of Ba^{2+}. d, the I-V relationship for EDHF obtained from 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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References

1. Adams DJ, Barakeh J, Laskey R, van Breemen C. Ion channels and regulation of intracellular calcium in vascular endothelial cells. *FASEB J.* 1989; **3**: 2389-400.
2. Cannell MB, Sage SO. Bradykinin-evoked changes in cytoplasmic calcium and membrane currents in cultured bovine pulmonary artery endothelial cells. *J. Physiol.* 1989; **419**: 555-68.
3. Yamanaka A, Ishikawa T, Goto K. Characterization of endothelium-dependent relaxation independent of NO and prostaglandins in guinea pig coronary artery. *J. Pharmacol. Exp. Ther.* 1998; **285**: 480-9.
4. Ghisdal P, Morel N. Cellular target of voltage and calcium-dependent K^+ channel blockers involved in EDHF-mediated responses in rat superior mesenteric artery. *Br. J. Pharmacol.* 2001; **134**: 1021-8.
5. Chen G, Suzuki H, Weston AH. Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. *Br. J. Pharmacol.* 1988; **95**: 1165-74.
6. Félétou M, Vanhoutte PM. Endothelium-dependent hyperpolarization of canine coronary smooth muscle. *Br. J. Pharmacol.* 1988; **93**: 515-24.
7. Taylor SG, Weston AH. Endothelium-derived hyperpolarizing factor: a new endogenous inhibitor from the vascular endothelium. *Trends Pharmacol. Sci.* 1988; **9**: 272-4.
8. McGuire J, Ding H, Triggle C. Endothelium-derived relaxing factors: a focus on endothelium-derived hyperpolarizing factors. *Can. J. Pharmacol.* 2001; **79**: 443-70.
9. Busse R, Edwards G, Félétou M, Fleming I, Vanhoutte PM, Weston AH. EDHF: bringing the concepts together. *Trends Pharmacol. Sci.* 2002; **23**: 374-80.
10. Little TL, Xia J, Duling BR. Dye tracers define differential endothelial and smooth muscle coupling patterns within the arteriolar wall. *Circ. Res.* 1995; **76**: 498-504.
11. Bény J-L. Electrical coupling between smooth muscle cells and endothelial cells in pig coronary arteries. *Pflügers Arch.* 1997; **433**: 364-7.
12. Chaytor AT, Evans WH, Griffith TM. Central role of heterocellular gap junctional communication in endothelium-dependent relaxations of rabbit arteries. *J. Physiol.* 1998; **508**: 561-73.
13. Yamamoto Y, Fukuta H, Nakahira Y, Suzuki H. Blockade by 18β-glycyrrhetinic acid of intercellular electrical coupling in guinea-pig arterioles. *J. Physiol.* 1998; **511**: 501-8.
14. Bény J-L. Information networks in the arterial wall. *News Physiol. Sci.* 1999; **14**: 68-73.
15. Edwards G, Félétou M, Gardener MJ, Thollon C, Vanhoutte PM, Weston AH. Role of gap junctions in the responses to EDHF in rat and guinea-pig small arteries. *Br. J. Pharmacol.* 1999; **128**: 1788-94.
16. Coleman HA, Tare M, Parkington HC. K^+ currents underlying the action of endothelium-derived

hyperpolarizing factor in guinea-pig, rat and human blood vessels. *J. Physiol.* 2001; **531**: 359-73.

17. Coleman HA, Tare M, Parkington HC. EDHF is not K^+ but may be due to spread of current from the endothelium in guinea pig arterioles. *Am. J. Physiol.* 2001; **280**: H2478-83.
18. Ganitkevich VY, Isenberg G. Membrane potential modulates inositol 1,4,5-trisphosphate-mediated Ca^{2+} transients in guinea-pig coronary myocytes. *J. Physiol.* 1993; **470**: 35-44.
19. Itoh T, Seki N, Suzuki S, Ito S, Kajikuri J, Kuriyama H. Membrane hyperpolarization inhibits agonist-induced synthesis of inositol 1,4,5-trisphosphate in rabbit mesenteric artery. *J. Physiol.* 1992; **451**: 307-28.
20. Hecker M, Bara AT, Bauersachs J, Busse R. Characterization of endothelium-derived hyperpolarizing factor as a cytochrome P450-derived arachidonic acid metabolite in mammals. *J. Physiol.* 1994; **481**: 407-14.
21. Campbell WB, Gebremedhin D, Pratt PF, Harder DR. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. *Circ. Res.* 1996; **78**: 415-23.
22. Adeagbo ASO, Henzel MK. Calcium-dependent phospholipase A_2 mediates the production of endothelium-derived hyperpolarizing factor in perfused rat mesenteric prearteriolar bed. *J. Vasc. Res.* 1998; **35**: 27-35.
23. Fisslthaler B, Popp R, Kiss L, Potente M, Harder DR, Fleming I, et al. Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature* 1999; **401**: 493-7.
24. Campbell WB, Harder DR. Endothelium-derived hyperpolarizing factors and vascular cytochrome P450 metabolites of arachidonic acid in the regulation of tone. *Circ. Res.* 1999; **84**: 484-8.
25. Quilley J, McGiff JC. Is EDHF an epoxyeicosatrienoic acid? *Trends Pharmacol. Sci.* 2000; **21**: 121-4.
26. Edwards G, Thollon C, Gardener MJ, Félétou M, Vilaine JP, Vanhoutte PM, et al. Role of gap junctions and EETs in endothelium-dependent hyperpolarization of porcine coronary artery. *Br. J. Pharmacol.* 2000; **129**: 1145-54.
27. Fleming I. Cytochrome P-450 enzymes in vascular homeostasis. *Circ. Res.* 2001; **89**: 753-62.
28. Edwards G, Dora KA, Gardener MJ, Garland CJ, Weston AH. K^+ is an endothelium-derived hyperpolarizing factor in rat arteries. *Nature* 1998; **396**: 269-72.
29. Chen G, Suzuki H. Some electrical properties of the endothelium-dependent hyperpolarization recorded from rat arterial smooth muscle cells. *J. Physiol.* 1989; **410**: 91-106.
30. Chen G, Hashitani H, Suzuki H. Endothelium-dependent relaxation and hyperpolarization of canine coronary artery smooth muscles in relation to the electrogenic Na-K pump. *Br. J. Pharmacol.* 1989; **98**: 950-6.
31. Adeagbo ASO, Triggle CR. Varying extracellular $[K^+]$: A functional approach to separating EDHF- and EDNO-related mechanisms in perfused rat mesenteric arterial bed. *J. Cardiovasc. Pharmacol.* 1993; **21**: 423-9.
32. Cowan CL, Palacino JJ, Najibi S, Cohen RA. Potassium channel-mediated relaxation to acetylcholine in rabbit arteries. *J. Pharmacol. Exp. Ther.* 1993; **266**: 1482-9.
33. Waldron GJ, Garland CJ. Effect of potassium channel blockers on L-NAME insensitive relaxations in rat small mesenteric artery. *Can. J. Physiol. Pharmacol.* 1994; **72**: S1, 115.
34. Zygmunt PM, Högestätt ED. Role of potassium channels in endothelium-dependent relaxation resistant to nitroarginine in the rat hepatic artery. *Br. J. Pharmacol.* 1996; **117**: 1600-6.
35. Petersson J, Zygmunt PM, Hogestatt ED. Characterization of the potassium channels involved in EDHF-mediated relaxation in cerebral arteries. *Br. J. Pharmacol.* 1997; **120**: 1344-50.
36. Plane F, Holland M, Waldron GJ, Garland CJ, Boyle JP. Evidence that anandamide and EDHF act via different mechanisms in rat isolated mesenteric arteries. *Br. J. Pharmacol.* 1997; **121**: 1509-11.
37. Sunano S, Watanabe H, Tanaka S, Sekiguchi F, Shimamura K. Endothelium-derived relaxing, contracting and hyperpolarizing factors of mesenteric arteries of hypertensive and normotensive rats. *Br. J. Pharmacol.* 1999; **126**: 709-16.
38. Büssemaker E, Wallner C, Fisslthaler B, Fleming I. The Na-K-ATPase is a target for an EDHF displaying characteristics similar to potassium ions in the porcine renal interlobar artery. *Br. J. Pharmacol.* 2002; **137**: 647-54.
39. Nelli S, Wilson WS, Laidlaw H, Llano A, Middleton S, Price AG, et al. Evaluation of potassium ion as the endothelium-derived hyperpolarizing factor (EDHF) in the bovine coronary artery. *Br. J. Pharmacol.* 2003; **139**: 982-8.
40. Zygmunt PM, Edwards G, Weston AH, Larsson B, Hogestatt ED. Involvement of voltage-dependent potassium channels in the EDHF-mediated relaxation of rat hepatic artery. *Br. J. Pharmacol.* 1997; **121**: 141-9.
41. Nishikawa Y, Stepp DW, Chilian WM. In vivo location and mechanism of EDHF-mediated vasodilation in canine coronary microcirculation. *Am. J. Physiol.* 1999; **277**: H1252-9.
42. Huang A, Sun D, Smith CJ, Connetta JA, Shesely EG, Koller A, et al. In eNOS knockout mice skeletal muscle arteriolar dilation to acetylcholine is mediated by EDHF. *Am. J. Physiol.* 2000; **278**: H762-8.
43. Archer SL, Gragasin FS, Wu X, Wang S, McMurtry S, Kim DH, et al. Endothelium-derived hyperpolarizing factor in human internal mammary artery is 11,12-epoxyeicosatrienoic acid and causes

relaxation by activating smooth muscle BK_{Ca} channels. *Circ.* 2003; **107**: 769-76.

44. Rapacon M, Mieyal P, McGiff JC, Fulton D, Quilley J. Contribution of calcium-activated potassium channels to the vasodilator effect of bradykinin in the isolated, perfused kidney of the rat. *Br. J. Pharmacol.* 1996; **118**: 1504-8.
45. Eckman DM, Hopkins N, McBride C, Keef KD. Endothelium-dependent relaxation and hyperpolarization in guinea-pig coronary artery: role of epoxyeicosatrienoic acid. *Br. J. Pharmacol.* 1998; **124**: 181-9.
46. Fulton D, McGiff JC, Quilley J. Pharmacological evaluation of an epoxide as the putative hyperpolarizing factor mediating the nitric oxide-independent vasodilator effect of bradykinin in the rat heart. *J. Pharmacol. Exp. Ther.* 1998; **287**: 497-503.
47. Chataigneau T, Félétou M, Duhault J, Vanhoutte PM. Epoxyeicosatrienoic acids, potassium channel blockers and endothelium-dependent hyperpolarization in the guinea-pig carotid artery. *Br. J. Pharmacol.* 1998; **123**: 574-80.
48. Balduini W, Costa LG, Murphy SD. Potassium ions potentiate the muscarinic receptor-stimulated phosphoinositide metabolism in cerebral cortex slices: A comparison of neonatal and adult rats. *Neurochem. Res.* 1990; **15**: 33-9.
49. Parkington HC, Tonta MA, Coleman HA, Tare M. Role of membrane potential in endothelium-dependent relaxation of guinea-pig coronary arterial smooth muscle. *J. Physiol.* 1995; **484**: 469-80.
50. Hammarström AK, Parkington HC, Coleman HA. The effects of some ion channel blockers on the hyperpolarisation evoked by EDHF in the guinea pig coronary artery. *Proc. Aust. Physiol. Pharmacol. Soc.* 1994; **25**: 63P.
51. Nishiyama M, Hashitani H, Fukuta H, Yamamoto Y, Suzuki H. Potassium channels activated in endothelium-dependent hyperpolarization in guinea-pig coronary artery. *J. Physiol.* 1998; **510**: 455-65.
52. Corriu C, Félétou M, Canet E, Vanhoutte PM. Endothelium-derived factors and hyperpolarization of the carotid artery of the guinea-pig. *Br. J. Pharmacol.* 1996; **119**: 959-64.
53. Hashitani H, Suzuki H. K^+ channels which contribute to the acetylcholine-induced hyperpolarization in smooth muscle of the guinea-pig submucosal arteriole. *J. Physiol.* 1997; **501**: 319-29.
54. Coleman HA, Tare M, Parkington HC. Components of the potassium currents underlying the actions of endothelium-derived hyperpolarizing factor in arterioles. In: Vanhoutte PM, editor. EDHF 2000. London: Taylor & Francis, 2001:259-69.
55. Sandow SL, Bramich NJ, Bandi HP, Rummery NM, Hill CE. Structure, function, and endothelium-derived hyperpolarizing factor in the caudal artery of the SHR and WKY rat. *Arterioscler. Thromb. Vasc. Biol.* 2003; **23**: 822-8.
56. Chen G, Cheung DW. Effect of K^+-channel blockers on ACh-induced hyperpolarization and relaxation in mesenteric arteries. *Am. J. Physiol.* 1997; **272**: H2306-12.
57. Murphy ME, Brayden JE. Apamin-sensitive K^+ channels mediate an endothelium-dependent hyperpolarization in rabbit mesenteric arteries. *J. Physiol.* 1995; **489.3**: 723-34.
58. Wulff H, Miller MJ, Hänsel W, Grissmer S, Cahalan MD, Chandy KG. Design of a potent and selective inhibitor of the intermediate-conductance Ca^{2+}-activated K^+ channel, *IKCa1*: A potential immunosuppressant. *Proc. Natl. Acad. Sci. USA.* 2000; **97**: 8151-6.
59. Eichler I, Wibawa J, Grgic I, Knorr A, Brakemeier S, Pries AR, et al. Selective blockade of endothelial Ca^{2+}-activated small- and intermediate-conductance K^+-channels suppresses EDHF-mediated vasodilation. *Br. J. Pharmacol.* 2003; **138**: 594-601.
60. Hinton JM, Langton PD. Inhibition of EDHF by two new combinations of K^+-channel inhibitors in rat isolated mesenteric arteries. *Br. J. Pharmacol.* 2003; **138**: 1031-5.
61. Crane GJ, Gallagher N, Dora KA, Garland CJ. Small- and intermediate-conductance calcium-activated K^+ channels provide different facets of endothelium-dependent hyperpolarization in rat mesenteric artery. *J. Physiol.* 2003; **553**: 183-9.
62. Sandow SL, Goto K, Rummery NM, Hill CE. Developmental changes in myoendothelial gap junction mediated vasodilator activity in the rat saphenous artery. *J. Physiol.* 2004; **556**: 875-86.
63. Quignard JF, Félétou M, Thollon C, Vilaine JP, Duhault J, Vanhoutte PM. Potassium ions and endothelium-derived hyperpolarizing factor in guinea-pig carotid and porcine coronary arteries. *Br. J. Pharmacol.* 1999; **127**: 27-34.
64. Vanheel B, Van de Voorde J. Barium decreases endothelium-dependent smooth muscle responses to transient but not to more prolonged acetylcholine applications. *Pflügers Arch.* 1999; **439**: 123-9.
65. Drummond GR, Selemidis S, Cocks TM. Apamin-sensitive, non-nitric oxide (NO) endothelium-dependent relaxations to bradykinin in the bovine isolated coronary artery: no role for cytochrome P_{450} and K^+. *Br. J. Pharmacol.* 2000; **129**: 811-9.
66. Lacy PS, Pilkington G, Hanvesakul R, Fish HJ, Boyle JP, Thurston H. Evidence against potassium as an endothelium-derived hyperpolarizing factor in rat mesenteric small arteries. *Br. J. Pharmacol.* 2000; **129**: 605-11.
67. Buus NH, Simonsen U, Pilegaard HK, Mulvany MJ. Nitric oxide, prostanoid and non-NO, non-prostanoid involvement in acetylcholine relaxation of isolated human small arteries. *Br. J. Pharmacol.* 2000; **129**: 184-92.
68. Bény J-L, Schaad O. An evaluation of potassium ions as endothelium-derived hyperpolarizing factor in porcine coronary arteries. *Br. J. Pharmacol.* 2000;

131: 965-73.

69. Dora KA, Garland CJ. Properties of smooth muscle hyperpolarization and relaxation to K^+ in the rat isolated mesenteric artery. *Am. J. Physiol.* 2001; **280**: H2424-9.
70. Savage D, Perkins J, Lim CH, Bund SJ. Functional evidence that K^+ is the non-nitric oxide, non-prostanoid endothelium-derived relaxing factor in rat femoral arteries. *Vasc. Pharmacol.* 2003; **40**: 23-8.
71. Monteith GR, Blaustein MP. Different effects of low and high dose cardiotonic steroids on cytosolic calcium in spontaneously active hippocampal neurons and in co-cultured glia. *Brain Res.* 1998; **795**: 325-40.
72. Zhu Z, Tepel M, Neusser M, Zidek W. Low concentrations of ouabain increase cytosolic free calcium concentration in rat vascular smooth muscle cells. *Clinical Science* 1996; **90**: 9-12.
73. Korge P, Langer GA. Mitochondrial Ca^{2+} uptake, efflux, and sarcolemmal damage in Ca^{2+}-overloaded cultured rat cardiomyocytes. *Am. J. Physiol.* 1998; **274**: H2085-93.
74. Saxena NC, Fan JS, Tseng GN. Effects of elevating $[Na]_i$ on membrane currents of canine ventricular myocytes: role of intracellular Ca ions. *Cardiovasc. Res.* 1997; **33**: 548-60.
75. Martin PEM, Hill NS, Kristensen B, Errington RJ, Griffith TM. Ouabain exerts biphasic effects on connexin functionality and expression in vascular smooth muscle cells. *Br. J. Pharmacol.* 2004; **141**: 374-84.
76. Hirst GDS, Neild TO. Some properties of spontaneous excitatory junction potentials recorded from arterioles of guinea-pigs. *J. Physiol.* 1980; **303**: 43-60.
77. Finkel AS, Hirst GDS, Van Helden DF. Some properties of excitatory junction currents recorded from submucosal arterioles of guinea-pig ileum. *J. Physiol.* 1984; **351**: 87-98.
78. Neild TO. Measurement of arteriole diameter changes by analysis of television images. *Blood Vessels* 1989; **26**: 48-52.
79. Parkington HC, Tare M, Tonta MA, Coleman HA. Stretch revealed three components in the hyperpolarization of guinea-pig coronary artery in response to acetylcholine. *J. Physiol.* 1993; **465**: 459-76.
80. Coleman HA, Tare M, Parkington HC. Myoendothelial electrical coupling in arteries and arterioles and its implications for Endothelium-Derived Hyperpolarizing Factor. *Clin. Exp. Pharmacol. Physiol.* 2002; **29**: 630-7.
81. Sandow SL, Tare M, Coleman HA, Hill CE, Parkington HC. Involvement of myoendothelial gap junctions in the actions of endothelium-derived hyperpolarizing factor. *Circ. Res.* 2002; **90**: 1108-13.
82. Sandow SL, Hill CE. Incidence of myoendothelial gap junctions in the proximal and distal mesenteric arteries of the rat is suggestive of a role in endothelium-derived hyperpolarizing factor-mediated responses. *Circ. Res.* 2000; **86**: 341-6.
83. Neylon CB, Lang RJ, Fu Y, Bobik A, Reinhart PH. Molecular cloning and characterization of the intermediate-conductance Ca^{2+}-activated K^+ channel in vascular smooth muscle. *Circ. Res.* 1999; **85**: e33-43.
84. Köhler R, Wulff H, Eichler I, Kneifel M, Neumann D, Knorr A, et al. Blockade of the intermediate-conductance calcium-activated potassium channel as a new therapeutic strategy for restenosis. *Circ.* 2003; **108**: 1119-25.
85. Burnham MP, Bychov R, Félétou M, Richards GR, Vanhoutte PM, Weston AH, et al. Characterization of an apamin-sensitive small-conductance Ca^{2+}-activated K^+ channel in porcine coronary artery endothelium: relevance to EDHF. *Br. J. Pharmacol.* 2002; **135**: 1133-43.
86. Taylor MS, Bonev AD, Gross TP, Eckman DM, Brayden JE, Bond CT, et al. Altered expression of small-conductance Ca^{2+}-activated K^+ (SK3) channels modulates arterial tone and blood pressure. *Circ. Res.* 2003; **93**: 124-31.
87. Van Renterghem C, Vigne P, Frelin C. A charybdotoxin-sensitive, Ca^{2+}-activated K^+ channel with inward rectifying properties in brain microvascular endothelial cells: Properties and activation by endothelins. *J. Neurochem.* 1995; **65**: 1274-81.
88. Marchenko SM, Sage SO. Calcium-activated potassium channels in the endothelium of intact rat aorta. *J. Physiol.* 1996; **492**: 53-60.
89. Bychkov R, Burnham MP, Richards GR, Edwards G, Weston AH, Félétou M, et al. Characterization of a charybdotoxin-sensitive intermediate conductance Ca^{2+}-activated K^+ channel in porcine coronary endothelium: relevance to EDHF. *Br. J. Pharmacol.* 2002; **137**: 1346-54.
90. Chen G, Cheung DW. Characterization of acetylcholine-induced membrane hyperpolarization in endothelial cells. *Circ. Res.* 1992; **70**: 257-63.
91. Ohashi M, Satoh K, Itoh T. Acetylcholine-induced membrane potential changes in endothelial cells of rabbit aortic valve. *Br. J. Pharmacol.* 1999; **126**: 19-26.
92. Doughty JM, Plane F, Langton PD. Charybdotoxin and apamin block EDHF in rat mesenteric artery if selectively applied to the endothelium. *Am. J. Physiol.* 1999; **276**: H1107-12.
93. Widmann MD, Weintraub NL, Fudge JL, Brooks LA, Dellsperger KC. Cytochrome P-450 pathway in acetylcholine-induced canine coronary microvascular vasodilation in vivo. *Am. J. Physiol.* 1998; **274**: H283-9.
94. De Wit C, Esser N, Lehr H, Bolz S, Pohl U. Pentobarbital-sensitive EDHF comediates ACh-induced arteriolar dilation in the hamster microcirculation. *Am. J. Physiol.* 1999; **276**: H1527-34.

95. Hungerford JE, Sessa WC, Segal SS. Vasomotor control in arterioles of the mouse cremaster muscle. *FASEB J.* 2000; **14**: 197-207.
96. Parkington HC, Chow J-AM, Evans RG, Coleman HA, Tare M. Role for endothelium-derived hyperpolarizing factor in vascular tone in rat mesenteric and hindlimb circulations *in vivo*. *J. Physiol.* 2002; **542**: 929-37.
97. De Vriese AS, Van de Voorde J, Lameire NH. Effects of connexin-mimetic peptides on nitric oxide synthase- and cyclooxygenase-independent renal vasodilation. *Kidney Int.* 2002; **61**: 177-85.
98. Fujii K, Tominaga M, Ohmori S, Kobayashi K, Koga T, Takata Y, et al. Decreased endothelium-dependent hyperpolarization to acetylcholine in smooth muscle of the mesenteric artery of spontaneously hypertensive rats. *Circ. Res.* 1992; **70**: 660-9.
99. Kenny LC, Baker PN, Kendall DA, Randall MD, Dunn WR. Differential mechanisms of endothelium-dependent vasodilator responses in human myometrial small arteries in normal pregnancy and pre-eclampsia. *Clinical Science* 2002; **103**: 67-73.
100. Fukao M, Hattori Y, Kanno M, Sakuma I, Kitabatake A. Alterations in endothelium-dependent hyperpolarization and relaxation in mesenteric arteries from streptozotocin-induced diabetic rats. *Br. J. Pharmacol*. 1997; **121**: 1383-91.
101. Wigg SJ, Tare M, Tonta MA, O'Brien RC, Meredith IT, Parkington HC. Comparison of effects of diabetes mellitus on an EDHF-dependent and an EDHF-independent artery. *Am. J. Physiol.* 2001; **281**: H232-40.
102. Makino A, Ohuchi K, Kamata K. Mechanisms underlying the attenuation of endothelium-dependent vasodilatation in the mesenteric arterial bed of the streptozotocin-induced diabetic rat. *Br. J. Pharmacol.* 2000; **130**: 549-56.
103. De Vriese AS, Van de Voorde J, Blom HJ, Vanhoutte PM, Verbeke M, Lameire NH. The impaired renal vasodilator response attributed to endothelium-derived hyperpolarizing factor in streptozotocin-induced diabetic rats is restored by 5-methyltetrahydrofolate. *Diabetologia* 2000; **43**: 1116-25.
104. Lau WAK, Reid JJ. Comparison of endothelial cell function in large and small arteries from the obese zucker rat. *Proc. Aust. Physiol. Pharmacol. Soc.* 2000; **31**: 66P.
105. Pannirselvam M, Verma S, Anderson TJ, Triggle CR. Cellular basis of endothelial dysfunction in small mesenteric arteries from spontaneously diabetic (*db/db* -/-) mice: role of decreased tetrahydrobiopterin bioavailability. *Br. J. Pharmacol.* 2002; **136**: 255-63.
106. Tare M, Coleman HA, Parkington HC. Regulation of vascular smooth muscle relaxation by the endothelium in health and in diabetes (with a focus on endothelium-derived hyperpolarizing factor). *Neurophys.* 2003; **35**: 284-9.

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Changes in EDHF in hypertension and ageing: response to chronic treatment with renin-angiotensin system inhibitors

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Summary

1. Endothelial function is impaired in hypertension and ageing and this may be associated with an increase in cardiovascular disease. Several clinical studies have shown that blocking the renin-angiotensin system (RAS) improves endothelial function not only in hypertensive patients but also in normotensive patients with cardiovascular disease. The aim of the present study was to test whether endothelium-derived hyperpolarising factor (EDHF) -mediated smooth muscle hyperpolarisation and relaxation are altered in hypertension and ageing, and if so, whether chronic treatment with RAS inhibitors (the angiotensin-converting enzyme inhibitor enalapril and the angiotensin type 1 receptor antagonist candesartan) would correct such changes.

2. EDHF-mediated responses were examined in mesenteric arteries from 12-month-old spontaneously hypertensive rats (SHR) and 3-, 6-, 12-, and 24-month-old normotensive Wistar-Kyoto rats (WKY). Furthermore, both strains were treated for three months with either RAS blockers or a conventional therapy with hydralazine and hydrochlorothiazide from 9- to 12-month-old. In arteries of 12-month-old SHR, EDHF-mediated responses were impaired compared with age-matched WKY. In SHR, all the antihypertensive treatments improved the impairment of EDHF-mediated responses; however, RAS inhibitors tended to improve these responses to a greater extent compared with the conventional therapy with hydralazine and hydrochlorothiazide. In arteries of WKY, EDHF-mediated responses were impaired at the age of 12 and 24 months compared with those of 3- and 6-month-old rats, with the response tending to be impaired to a greater extent in 24-month-old rats. Three months of treatment of WKY until the age of 12 months with RAS inhibitors but not with a conventional therapy with hydralazine and hydrochlorothiazide improved the age-related impairment of EDHF-mediated responses, despite a similar reduction in blood pressure by both treatments.

3. These findings suggest that: (1) EDHF-mediated hyperpolarisation and relaxation decline with hypertension and ageing in rat mesenteric arteries; (2) antihypertensive treatment restores the impaired EDHF-mediated responses in hypertension; (3) RAS inhibitors may be more efficacious in improving endothelial dysfunction associated with hypertension; and (4) chronic treatment with RAS inhibitors improves the age-related impairment of EDHF-mediated responses presumably through the blockade of RAS but not blood pressure lowering alone.

Introduction

Endothelial cells play an important role in the regulation of vascular tone through the release of several factors such as nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarising factor (EDHF).[1,2] Although the nature of EDHF is still controversial, EDHF appears to be a dominant vasodilator in resistance arteries.[3-5]

Endothelial dysfunction is associated with various cardiovascular risk factors, such as hypertension, ageing, diabetes mellitus, and hypercholesterolemia.[6,7] Endothelial dysfunction may facilitate the progress of atherosclerosis[6,7], thereby leading to cardiovascular diseases.[8] It is, therefore, of clinical importance to find out the underlying mechanisms of, and effective treatments for endothelial dysfunction. In the present paper, the role of EDHF in hypertension and ageing and its modulation by drug treatment – especially the effects of renin-angiotensin system (RAS) inhibitors – will be discussed.

EDHF in hypertension

Endothelium-dependent relaxation is impaired both in animal models of experimental hypertension and in patients with hypertension.[9] Several mechanisms have been proposed to explain the endothelial dysfunction in hypertension: reduced NO production, increased production of endothelium-derived contracting factors and increased generation of oxygen-derived free radicals.[9]

Fujii *et al.*[10] have evaluated the relative contribution of EDHF in acetylcholine (ACh) -induced responses in the superior mesenteric arteries of spontaneously hypertensive rats (SHR). In this study, they showed that EDHF-mediated hyperpolarisation and relaxation were decreased in SHR compared with age-matched normotensive Wistar-Kyoto rats (WKY). In contrast, endothelium-dependent relaxation via NO was preserved in SHR.[11] Fujii *et al.* have also showed that neither NO synthase inhibitors nor a cyclooxygenase inhibitor affected ACh-induced hyperpolarisation in the rat superior mesenteric arteries,[10] which suggests that ACh-induced hyperpolarisation is not mediated by endothelium derived NO or prostanoids in this vascular bed. Subsequent studies[11-14] confirmed the impairment of EDHF-mediated responses in mesenteric arteries from genetically hypertensive rats. Similar

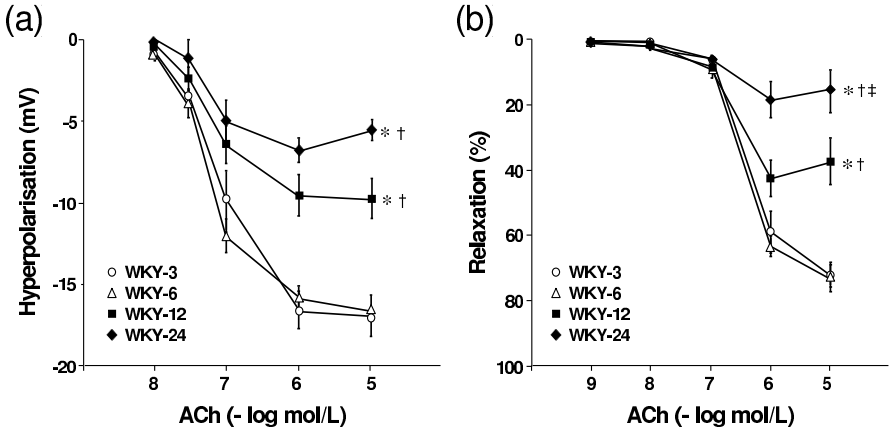

Figure 1. *(a) Acetylcholine (ACh)-induced hyperpolarisation in mesenteric arteries of 3- (WKY-3), 6- (WKY-6), 12- (WKY-12), and 24-month-old Wistar Kyoto rats (WKY-24). ACh was applied under resting conditions without treatment. (b) ACh-induced relaxation in mesenteric arterial rings precontracted with norepinephrine (10^{-5} mol/L) in the presence of indomethacin (10^{-5} mol/L) and N^G-nitro-L-arginine (10^{-4} mol/L) of WKY-3, WKY-6, WKY-12, and WKY-24. Values are mean±SEM (n=6-10). *P<0.05 vs. WKY-3; †P<0.05 vs. WKY-6; ‡P<0.05 vs. WKY-12. (Reproduced from Fujii* et al.,[21] *with permission).*

observations were also reported in the aorta of two-kidney, one clip renal hypertensive rats[15] and in the renal artery of aged SHR.[16] These findings indicate that EDHF-mediated responses are impaired in hypertension, and the impairment of EDHF pathway may account, at least in part, for the endothelial dysfunction associated with hypertension. On the other hand, it has been recently reported that enhanced EDHF effect may compensate for the loss of NO and maintain the vasodilatory response to ACh in mesenteric arteries of Sprague-Dawley rats fed a high salt diet.[17] Furthermore, Sandow *et al.* have reported that the incidence of myoendothelial gap junctions, which enables electrical and/or chemical coupling between endothelial cell and smooth muscle cell layers, was increased to maintain a functional role for EDHF in caudal artery of SHR.[18]

The reason for the difference in the results of these studies is not known, but may in part arise from differences in the type, severity and/or duration of hypertension.

EDHF in ageing

Ageing is associated with endothelial dysfunction both in humans and animal models.[19] Reduced NO-mediated relaxation and/or increased cyclooxygenase-dependent constriction could partially underpin age-related endothelial dysfunction depending on the species and the vascular bed studied.[19] In the present study, age related changes in EDHF-mediated hyperpolarisation and relaxation to ACh were studied in the superior mesenteric arteries from 3-, 6-, 12-, and 24-month-old WKY.[20,21] EDHF-mediated hyperpolarisation was significantly smaller in arteries from 12- and 24-month-old rats compared with 3- and 6-month-old rats, with the response tending to be smaller in 24-month-old rats than in 12-month-old rats. EDHF-mediated relaxation also decreased with increasing age (Fig. 1). In contrast, there was no difference in NO-mediated relaxation between 3- and 12-month-old rats. The age-related decline in EDHF-mediated responses observed here are consistent with previous studies by others.[12,22] Thus, the impairment of the EDHF pathway may account, at least in part, for the age-related endothelial dysfunction in rat mesenteric arteries.

The EDHF pathway does exist in human arteries.[23,24] Urakami-Harasawa *et al.*[24] have reported that EDHF-mediated relaxation was reduced with ageing in human gastroepiploic arteries. Thus, the reduced EDHF-mediated responses would also contribute to the age-related endothelial dysfunction in humans.

Effect of antihypertensive treatment on EDHF-mediated responses in hypertension

Hypertension is associated with endothelial dysfunction.[9] Endothelial dysfunction may aggravate the progression of atherosclerosis, which could lead to cardiovascular disease.[6-8] Hence, it is plausible to suggest that the improvement of endothelial function will reduce the occurrence of cardiovascular disease. Although several studies found that antihypertensive treatments improve endothelial function both in animal models of experimental hypertension[9] and in patients with hypertension,[25] the effects of chronic antihypertensive treatment on EDHF-mediated hyperpolarisation *per se* are unknown.

The effects of chronic antihypertensive treatments on EDHF-mediated hyperpolarisation and relaxation were tested in the mesenteric arteries of SHR.[11,14] SHR were

Table. *Systolic blood pressure before and after 3 months of treatment. Values are mean ± SEM. There were 7 to 12 rats in each group.*

	Blood pressure (mmHg)				Blood pressure (mmHg)	
	Before	After			Before	After
SHR-12	241±6	253±6		WKY-12	150±4	156±5
SHR-12-H	242±6	163±6 *†		WKY-12-H	158±4	124±4 *§
SHR-12-ENA	245±5	135±6 *†		WKY-12-ENA	157±3	123±6 *§
SHR-12-CAN	239±7	120±6 *†‡		WKY-12-CAN	153±3	125±2 *§
SHR-12-C&E	246±7	111±3 *†‡				
WKY-12	151±5 †	155±4 †				

* $P<0.05$ vs before treatment; † $P<0.05$ vs SHR-12; ‡ $P<0.05$ vs SHR-12-H; § $P<0.05$ vs WKY-12

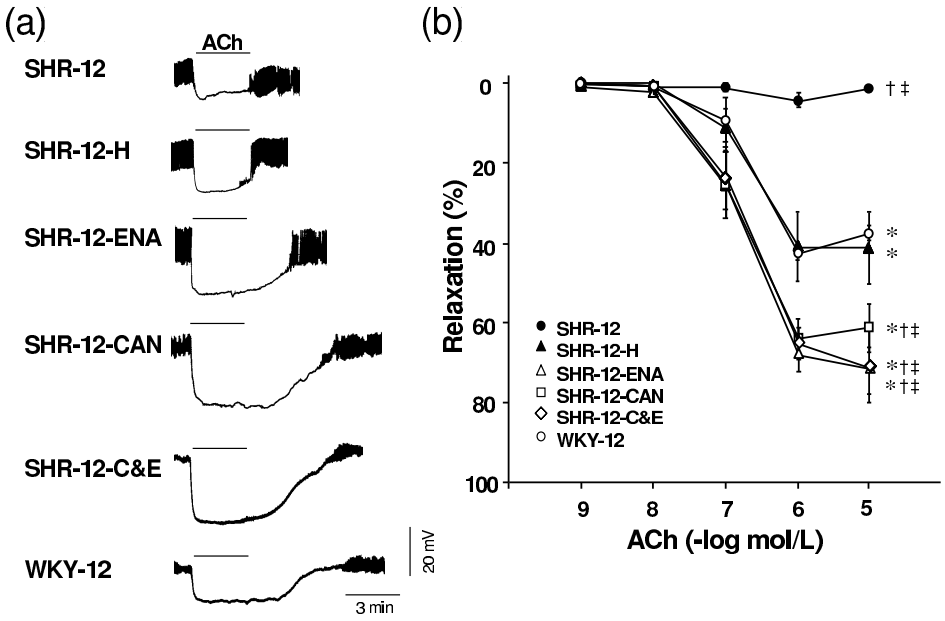

Figure 2. *(a) Representative tracings showing hyperpolarisation to 10^{-5} mol/L acetylcholine (ACh) under conditions of depolarisation with norepinephrine (10^{-5} mol/L) in the presence of indomethacin (10^{-5} mol/L) in mesenteric arteries of untreated 12-month-old spontaneously hypertensive rats (SHR-12), SHR treated with a combination of hydralazine and hydrochlorothiazide (SHR-12-H), enalapril-treated SHR (SHR-12-ENA), candesartan-treated SHR (SHR-12-CAN), SHR treated with a combination of candesartan and enalapril (SHR-12-C&E), and untreated 12-month-old Wistar Kyoto rats (WKY-12). (b) ACh-induced relaxation in mesenteric arterial rings precontracted with norepinephrine (10^{-5} mol/L) in the presence of indomethacin (10^{-5} mol/L) and N^G-nitro-L-arginine (10^{-4} mol/L) of SHR-12, SHR-12-H, SHR-12-ENA, SHR-12-CAN, SHR-12-C&E, and WKY-12. Values are mean±SEM (n=8-12). *P<0.05 vs. SHR-12; †P<0.05 vs. WKY-12; ‡P<0.05 vs. SHR-12-H. (Modified from Goto* et al.,[14] *with permission).*

treated for 3 months with either the combination of hydralazine and hydrochlorothiazide, enalapril, an angiotensin converting enzyme (ACE) inhibitor, candesartan, an angiotensin type 1 (AT1) receptor antagonist, or the combination of enalapril and candesartan from 9- to 12-month-old. The combination of hydralazine and hydrochlorothiazide improved EDHF-mediated hyperpolarisation and relaxation to a similar level to that of WKY. Interestingly, however, the improvement achieved by RAS inhibitors was significantly greater than that with a conventional therapy with hydralazine and hydrochlorothiazide, despite a similar, or only a slightly greater reduction in blood pressure (Table, Fig. 2). These results suggest that in addition to blood pressure lowering, inhibition of the RAS may play an important role in improving endothelial function.[11,14]

Although both ACE inhibitors and AT1 receptor antagonists inhibit the RAS, each drug has its specific

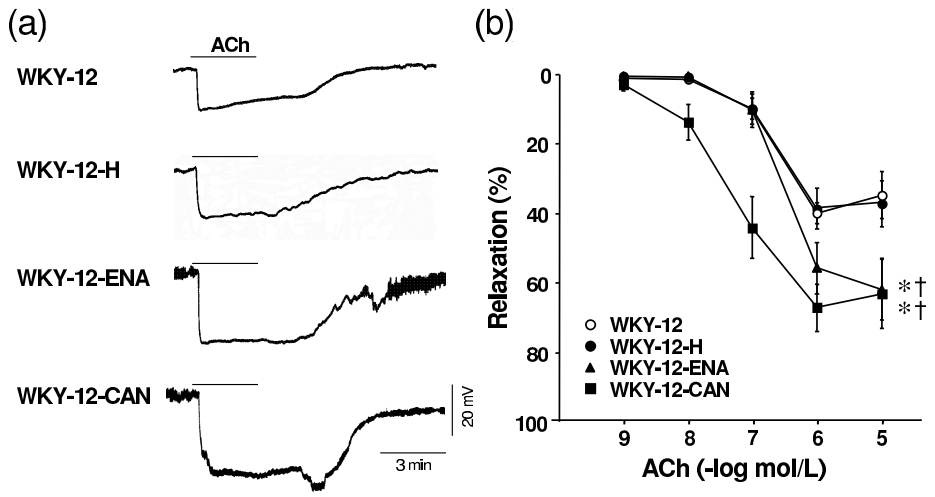

Figure 3. *(a) Representative tracings showing hyperpolarisation to 10^{-5} mol/L acetylcholine (ACh) under conditions of depolarisation with norepinephrine (10^{-5} mol/L) in the presence of indomethacin (10^{-5} mol/L) in mesenteric arteries of untreated 12-month-old Wistar Kyoto rats (WKY-12), WKY treated with a combination of hydralazine and hydrochlorothiazide (WKY-12-H), enalapril-treated WKY (WKY-12-ENA), and candesartan-treated WKY (WKY-12-CAN). (b) ACh-induced relaxation in mesenteric arterial rings precontracted with norepinephrine (10^{-5} mol/L) in the presence of indomethacin (10^{-5} mol/L) and N^G-nitro-L-arginine (10^{-4} mol/L) of WKY-12, WKY-12-H, WKY-12-ENA, and WKY-12-CAN. Values are mean±SEM (n=6-12). * P<0.05 vs. WKY-12; † P<0.05 vs. WKY-12-H. (Modified from Goto* et al.,[31] *with permission).*

pharmacological profiles: ACE inhibitors prevent the degradation of bradykinin, a peptide that induces endothelium-dependent relaxation[23]; AT1 receptor antagonists block the action of angiotensin II regardless of its generation pathway[26]; under blockade of AT1 receptors, angiotensin II may stimulate unopposed angiotensin type 2 receptors.[27] However, in the present study, enalapril and candesartan were equally effective in improving EDHF-mediated responses, which indicates that the specific pharmacological profiles of each drug may not play a major role in improving EDHF-mediated responses in rat mesenteric arteries. Kähönen *et al.*[28] also showed that an ACE inhibitor and an AT1 receptor antagonist improved the EDHF-mediated relaxation to a similar extent in mesenteric arteries of SHR.

Several recent clinical studies[29,30]have reported the beneficial effects of the combination therapy with an ACE inhibitor and an AT1 receptor antagonist. In the present study, however, the combination therapy did not appear to have definitive advantages over each therapy in improving EDHF-mediated responses (Fig. 2).

In summary, the above data indicate that: (1) chronic antihypertensive treatments restore the impaired EDHF-mediated responses in SHR; (2) RAS inhibitors may be more effective in improving endothelial dysfunction; and (3) the combination of an ACE inhibitor and an AT1 receptor antagonist does not seem to be more effective than treatment with either drug alone. The clinical relevance of the present finding remains to be determined.

Effect of renin-angiotensin system inhibitors on EDHF-mediated responses in ageing

Endothelial dysfunction associated with ageing may contribute in part to the frequent occurrence of cardiovascular disease with ageing in humans. Thus, it is clinically relevant to prevent or reverse endothelial dysfunction associated with ageing. In SHR, antihypertensive treatments with RAS inhibitors tended to be more effective in improving endothelial dysfunction compared with conventional antihypertensive drugs.[11,14] These observations led to the hypothesis that RAS inhibitors may have a favourable effect on endothelial function independent of its blood pressure lowering effect.

The effects of RAS inhibitors on age-related impairment of EDHF-mediated responses were studied using mesenteric arteries of WKY.[31,32] WKY were treated for 3 months with either enalapril, candesartan or a combination of hydralazine and hydrochlorothiazide from 9- to 12-month-old. All the treatments lowered blood pressure to a similar extent (Table). EDHF-mediated hyperpolarisation and relaxation were improved in the enalapril and candesartan treated groups. In contrast, a combination of hydralazine and hydrochlorothiazide failed to improve endothelial function, despite a similar reduction in blood pressure (Fig. 3). These findings suggest that RAS inhibitors restore the age-related impairment of EDHF-mediated responses presumably through the blockade of the RAS *per se*, although we cannot totally rule out the possibility that both RAS inhibition and blood pressure

lowering are required for the improvement of endothelial function. Thus, RAS inhibitors may serve as novel tools with which to prevent endothelial dysfunction associated with ageing.

Future directions

Because of the unidentified nature of EDHF,[3-5] the mechanism of the alteration in EDHF associated with hypertension and ageing remains speculative. Likewise, how RAS inhibitors improve impaired EDHF-mediated responses remains an open question. However, considering the critical role of gap junctions in EDHF-mediated responses in rat mesenteric arteries[33,34], impairment of the EDHF pathway and its improvement by RAS inhibitors could be associated with structural and/or biochemical changes in gap junctions. This notion may be supported by the recent report by Rummery *et al.*[35]that showed expression of connexins, which comprise gap junctions, were decreased in the endothelium of the caudal artery in hypertension. Whether impairment of EDHF-mediated responses in disease states is attributable to abnormalities of gap junctions awaits further studies.

Conclusions

EDHF mediated hyperpolarisation and relaxation were impaired in hypertension and ageing. Chronic treatment with RAS inhibitors restored these impairments, and RAS inhibitors appear to have a favourable effect on endothelial function beyond its blood pressure lowering effect. Thus, RAS inhibitors may have a therapeutic potential in the prevention or treatment of cardiovascular diseases.

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References

1. Hill CE, Phillips JK, Sandow SL. Heterogeneous control of blood flow amongst different vascular beds. *Med. Res. Rev.* 2001; **21**: 1-60.
2. Suzuki H, Chen G. Endothelium-derived hyperpolarizing factor (EDHF): An endogenous potassium-channel activator. *News Physiol. Sci.* 1990; **5**: 212-5.
3. McGuire JJ, Ding H, Triggle CR. Endothelium-derived relaxing factors: a focus on endothelium-derived hyperpolarizing factor(s). *Can. J. Physiol. Pharmacol.* 2001; **79**: 443-70.
4. Campbell WB, Gauthier KM. What is new in endothelium-derived hyperpolarizing factors? *Curr. Opin. Nephrol. Hypertens.* 2002; **11**: 177-83.
5. Busse R, Edwards G, Feletou M, Fleming I, Vanhoutte PM, Weston AH. EDHF: bringing the concepts together. *Trends Pharmacol. Sci.* 2002; **23**: 374-80.
6. Vanhoutte PM. Endothelial dysfunction and atherosclerosis. *Eur. Heart J.* 1997; **18**: E19-29.
7. Schiffrin EL. Beyond blood pressure: the endothelium and atherosclerosis progression. *Am. J. hypertens.* 2002; **15**: 115S-22S.
8. Halcox JP, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, Nour KR, Quyyumi AA**.** Prognostic value of coronary vascular endothelial dysfunction. *Circulation* 2002; **106**: 653-8.
9. Vanhoutte PM. Endothelial dysfunction in hypertension. *J. Hypertens.* 1996; **14**: S83-93.
10. Fujii K, Tominaga M, Ohmori S, Kobayashi K, Koga T, Takata Y, Fujishima M. Decreased endothelium-dependent hyperpolarization to acetylcholine in smooth muscle of the mesenteric artery of spontaneously hypertensive rats. *Circ. Res.* 1992; **70**: 660-9.
11. Onaka U, Fujii K, Abe I, Fujishima M. Antihypertensive treatment improves endothelium-dependent hyperpolarization in the mesenteric artery of spontaneously hypertensive rats. *Circulation* 1998; **98**: 175-82.
12. Mantelli L, Amerini S, Ledda F. Roles of nitric oxide and endothelium-derived hyperpolarizing factor in vasorelaxant effect of acetylcholine as influenced by aging and hypertension. *J. Cardiovasc. Pharmacol.* 1995; **25**: 595-602.
13. Sunano S, Watanabe H, Tanaka S, Sekiguchi F, Shimamura K. Endothelium-derived relaxing, contracting and hyperpolarizing factors of mesenteric arteries of hypertensive and normotensive rats. *Br. J. Pharmacol.* 1999; **126**: 709-16.
14. Goto K, Fujii K, Onaka U, Abe I, Fujishima M. Renin-angiotensin system blockade improves endothelial dysfunction in hypertension. *Hypertension* 2000; **36**: 575-80.
15. Van de Voorde J, Vanheel B, Leusen I. Endothelium-dependent relaxation and hyperpolarization in aorta from control and renal hypertensive rats. *Circ. Res.* 1992; **70**: 1-8.
16. Bussemaker E, Popp R, Fisslthaler B, Larson CM, Fleming I, Busse R, Brandes RP. Aged spontaneously hypertensive rats exhibit a selective loss of EDHF-mediated relaxation in the renal artery. *Hypertension* 2003; **42**: 562-8**.**
17. Sofola OA, Knill A, Hainsworth R, Drinkhill M. Change in endothelial function in mesenteric arteries of Sprague-Dawley rats fed a high salt diet. *J. Physiol.* 2002; **543**: 255-60.
18. Sandow SL, Bramich NJ, Bandi HP, Rummery NM, Hill CE. Structure, function, and endothelium-derived hyperpolarizing factor in the caudal artery of the SHR and WKY rat. *Arterioscler. Thromb. Vasc.*

Biol. 2003; **23**: 822-8.
19. Matz RL, Andriantsitohaina R. Age-related endothelial dysfunction: potential implications for pharmacotherapy. *Drugs Aging.* 2003; **20**: 527-50.
20. Fujii K, Ohmori S, Tominaga M, Abe I, Takata Y, Ohya Y, Kobayashi K, Fujishima M. Age-related changes in endothelium-dependent hyperpolarization in the rat mesenteric artery. *Am. J. Physiol.* 1993; **265**: H509-16.
21. Fujii K, Goto K, Abe I. Inhibition of converting enzyme prevents the age-related decline in endothelium-dependent hyperpolarization. In: Vanhoutte PM (ed). EDHF 2000. Taylor & Francis, London. 2001; Ch. 45.
22. Nakashima M, Vanhoutte PM. Decreased endothelium-dependent hyperpolarization with aging and hypertension in the rat mesenteric artery. In: Vanhoutte PM (ed). Endothelium-Derived Hyperpolarizing Factor. Harwood Academic, Amsterdam. 1996; Ch. 28.
23. Nakashima M, Mombouli JV, Taylor AA, Vanhoutte PM. Endothelium-dependent hyperpolarization caused by bradykinin in human coronary arteries. *J. Clin. Invest.* 1993; **92**: 2867-71.
24. Urakami-Harasawa L, Shimokawa H, Nakashima M, Egashira K, Takeshita A. Importance of endothelium-derived hyperpolarizing factor in human arteries. *J. Clin. Invest.* 1997; **100**: 2793-9.
25. Taddei S, Virdis A, Ghiadoni L, Sudano I, Salvetti A. Effects of antihypertensive drugs on endothelial dysfunction: clinical implications. *Drugs* 2002; **62**: 265-84.
26. Urata H, Nishimura H, Ganten D. Chymase-dependent angiotensin II forming systems in humans. *Am. J. Hypertens.* 1996; **9**: 277-84.
27. Matsubara H. Pathophysiological role of angiotensin II type 2 receptor in cardiovascular and renal diseases. *Circ. Res.* 1998; **83**: 1182-91.
28. Kahonen M, Tolvanen JP, Kalliovalkama J, Wu X, Karjala K, Makynen H, Porsti I. Losartan and enalapril therapies enhance vasodilatation in the mesenteric artery of spontaneously hypertensive rats. *Eur. J. Pharmacol.* 1999; **368**: 213-22.
29. McKelvie RS, Yusuf S, Pericak D, Avezum A, Burns RJ, Probstfield J, Tsuyuki RT, White M, Rouleau J, Latini R, Maggioni A, Young J, Pogue J. Comparison of candesartan, enalapril, and their combination in congestive heart failure: randomized evaluation of strategies for left ventricular dysfunction (RESOLVD) pilot study. The RESOLVD Pilot Study Investigators. *Circulation* 1999; **100**: 1056-64.
30. McMurray JJ, Ostergren J, Swedberg K, Granger CB, Held P, Michelson EL, Olofsson B, Yusuf S, Pfeffer MA**;** CHARM Investigators and Committees. Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function taking angiotensin-converting-enzyme inhibitors: the CHARM-Added trial. *Lancet* 2003; **362**: 767-71.
31. Goto K, Fujii K, Onaka U, Abe I, Fujishima M. Angiotensin-converting enzyme inhibitor prevents age-related endothelial dysfunction. *Hypertension* 2000; **36**: 581-7.
32. Kansui Y, Fujii K, Goto K, Abe I, Iida M. Angiotensin II receptor antagonist improves age-related endothelial dysfunction. *J. Hypertens.* 2002; **20**: 439-46.
33. Goto K, Fujii K, Kansui Y, Abe I, Iida M. Critical role of gap junctions in endothelium-dependent hyperpolarization in rat mesenteric arteries. *Clin. Exp. Pharmacol. Physiol.* 2002; **29**: 595-602.
34. Sandow SL, Tare M, Coleman HA, Hill CE, Parkington HC. Involvement of myoendothelial gap junctions in the actions of endothelium-derived hyperpolarizing factor. *Circ. Res.* 2002; **90**: 1108-13.
35. Rummery NM, McKenzie KU, Whitworth JA, Hill CE. Decreased endothelial size and connexin expression in rat caudal arteries during hypertension. *J. Hypertens*. 2002; **20**: 247-53..br

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