

# Implications for Endothelium-Derived Hyperpolarizing Factor (EDHF) in Women's Cardiovascular Health

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**Abstract:** Several clinical conditions related to women's cardiovascular health strongly correlates with endothelial dysfunction, which is conventionally associated with alterations in synthesis, release or bioavailability of endothelium-derived nitric oxide (NO). Current review pays attention to rapidly growing evidence about the importance of Endothelium-Derived Hyperpolarizing Factor (EDHF). EDHF-mediated action is pertinent to resistance circulation where EDHF overcomes NO contribution, while in large conduit vessels endothelium-dependent dilatation is predominantly conferred by NO. This indicates that changes in synthesis, release, or pharmacological manipulation of EDHF is of critical importance in the maintenance of organ perfusion, peripheral resistance and blood pressure, the disturbances in which distinctively predispose the development of cardiovascular disorders. This review describes current knowledge about EDHF, including nature and characterization of its action, alterations in the mechanisms of EDHF contribution to endothelium-dependent relaxation with particular focus on preeclampsia, gender differences and cardiovascular complications after menopause. The distinction in the relative contribution of NO versus EDHF and estrogen-related regulation of EDHF-mediated responses are highlighted. The indications that EDHF-mediated response accounts for different chemical mediator or electrical transmission depending on species, vascular bed and healthy or diseased condition are discussed paying attention to women's cardiovascular health and future therapeutic implications.

**Keywords:** EDHF, endothelium, estrogen, pregnancy, preeclampsia.

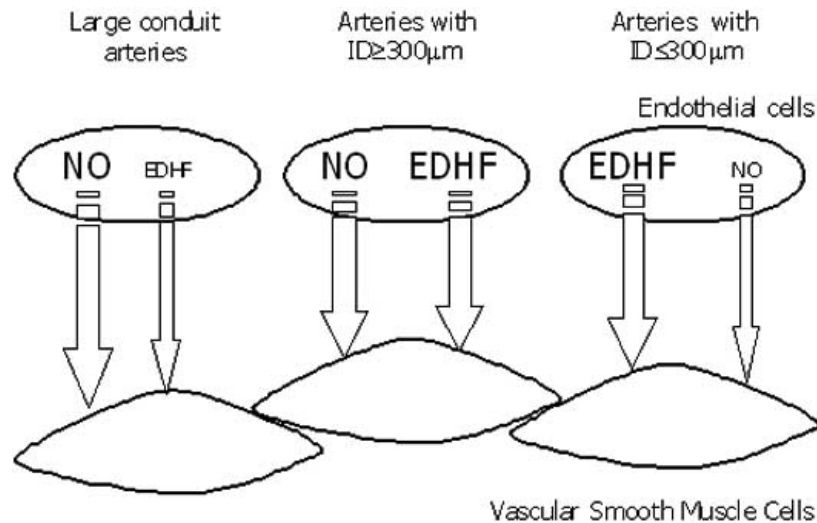
## INTRODUCTION

About a quarter of century has passed since classical study about the vital importance of endothelium on vasomotor control has been recognized [1]. PGI<sub>2</sub> – a cyclooxygenase-dependent metabolite of arachidonic acid [2] and NO formed through L-arginine and NO synthase (NOS) pathway [3] have been identified as major endothelium-derived vasodilators. However, the evidence that NO and PGI<sub>2</sub> could not fully account for the agonist-induced relaxation in certain circulations (i.e. resistance vasculature), as endothelium-dependent relaxation was not dramatically affected by the pharmacological inhibition of NO and PGI<sub>2</sub> production, suggested the existence of an additional endothelium-dependent but NO and PGI<sub>2</sub> independent vasodilative mechanism [4-6]. Since the residual endothelium-dependent relaxation was concurrent with vascular smooth muscle cells (VSMC) hyperpolarization [7] and abolished by potassium channel blockers or depolarizing concentration of potassium (an increase extracellular K<sup>+</sup> from the basal 4.7 to additional 25-30 mM) [8], the mediator responsible for this occurrence was termed as endothelium-derived hyperpolarizing factor (EDHF) [9]. Thus, by definition, the EDHF seems to be a substance and/or electrical signal that is generated or synthesized in and released from the endothelium that hyperpolarizes VSMC with followed relaxation without an increase in intracellular levels of cyclic nucleotides [10].

Although the nature and mechanism of EDHF action is wrapped in a shroud of mystery, the importance of EDHF-mediated responses is determined by their predominant contribution to endothelium-dependent modulation of underlying VSMC tone in resistance-sized arteries [11, 12]. Indeed, evidence exists that EDHF-mediated contribution to endothelium-dependent dilatation increases as the vessel size became smaller [13, 14]. If NO and PGI<sub>2</sub> inhibitors almost fully prevent endothelium-dependent relaxation in conduit arteries [15, 16], the dilative capacity of endothelium is about equally divided between EDHF and NO in vessels at the diameter not less than 300µm [17]. When the diameter decreases less than 300µm the contribution of EDHF increases, whereas the role of NO remains minimal [18, 19] (Fig. 1). This functional evidence well correlates with electrophysiological experiments, where endothelium-dependent changes in membrane potential are more pronounced in smaller compared to large arteries [13]. In line, an inverse relationship between eNOS expression and vessel size in aorta versus proximal and distal mesenteric arteries has been estimated [20].

Since EDHF appears to be most important in the small arteries it might be suggested that changes in the synthesis and/or release of EDHF is of critical significance for the regulation of organ blood flow, peripheral vascular resistance and blood pressure, especially under the circumstances of compromised NO production. Furthermore, depending on the type of cardiovascular disorder, the altered EDHF responses may contribute to [21, 22], or compensate for [23, 24] the endothelial dysfunction associated with pathogenesis of several diseases. Consequently, identification of vessel-specific nature of EDHF, and selective

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**Fig. (1).** Generalized schematic representation of the balance between agonist-induced NO and EDHF release in arteries depending on the internal diameter (ID).

activators or inhibitors of its biological activity, might have a significant impact to our understanding of vascular pathophysiology and provide the basis for the design of novel therapeutic alternatives [25].

Considering the importance of resistance vasculature in the maintenance of blood supply requirements to target organs, the prevalence of EDHF-mediated responses in small arteries plays a meticulous biological role. The accessibility of EDHF-mediated mechanism for endothelium-dependent relaxation in addition to NO- and PGI<sub>2</sub>-mediated dilatation may provide a “factor of safety” to preserve vasodilative capacity of the endothelium in resistance circulation where endothelium-dependent relaxation appears to be of vital importance. A distinct nature of EDHF, as demonstrated in the same arteries or circulations by different research groups, may also reflect a flexibility of the mechanism/s responsible for EDHF-mediated relaxation, which depends on physiological or pathophysiological state of the organism.

In this paper we will summarize the current knowledge based on experimental evidence about EDHF, including nature and characterization of its action, alterations in the mechanisms of EDHF contribution to endothelium-dependent relaxation with particular focus on female's resistance circulation and possible implications for cardiovascular health. Female's cardiovascular system undergoes significant changes during normal pregnancy to meet the demands of growing fetus, while in preeclampsia failure of this adaptation and alteration in endothelium-dependent dilatation may have acute and long-term adverse effects on women's cardiovascular health. Moreover, the female sex hormone estrogen plays an important role in gender-specific regulation of endothelial function and it is responsible for cardiovascular complications during menopause.

#### MECHANISMS OF EDHF RELEASE AND ACTION

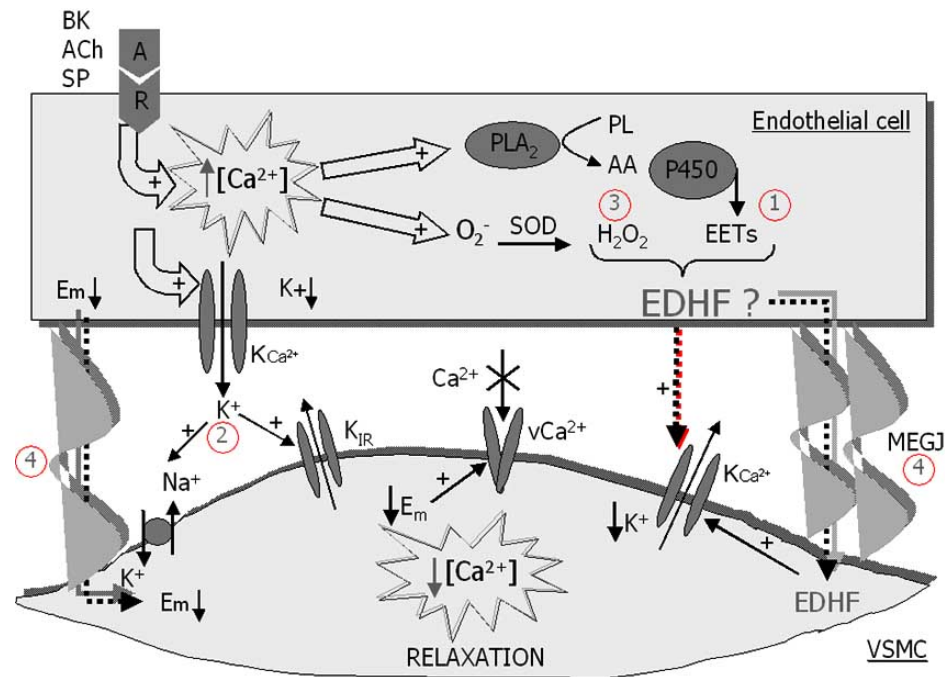
The evidence that agonist acetylcholine (ACh) may cause hyperpolarization of VSMC has been reported before the recognition of the importance of the endothelium to

dilatation [26]. The final agreement that agonist-induced hyperpolarization occurs through a release of a factor named EDHF has been reached a few years later after the confirmation of endothelium-dependent dilatation [27]. The overall picture of EDHF release or generation of hyperpolarizing signal within the endothelial cell (EC) and response of VSMC to induce EDHF-mediated relaxation is presented in (Fig. 2).

The basic mechanism of EDHF-mediated response may be separated into two stages based on the place in which the events are taken place. An increase in intracellular Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>i</sub>), activation of Ca<sup>2+</sup>-dependent K<sup>+</sup>-channels (K<sub>Ca</sub>) and K<sup>+</sup> efflux followed by hyperpolarization, synthesis of substance or generation of signal capable to diffuse through membranes or myoendothelial gap junctions (MEGJ) to VSMC confer endothelial stage of EDHF-mediated response. The following stage reflects the mechanism by which EC hyperpolarization is transferred to VSMC. At the level of VSMC, EDHF activates K<sup>+</sup>-channels and causes endothelium-dependent hyperpolarization (EDH) accompanied by closure of voltage-sensitive Ca<sup>2+</sup>-channels that results in relaxation [11, 28].

The elevation of [Ca<sup>2+</sup>]<sub>i</sub> in EC is a critical event for the synthesis or release of EDHF [29], however the relative contribution of either transmembrane Ca<sup>2+</sup> influx or Ca<sup>2+</sup> release from intracellular sources remains unsettled issue. It might be suggested that emptying the intracellular stores of Ca<sup>2+</sup> is a triggering pathway to initiate EDHF production, while transmembrane Ca<sup>2+</sup> influx through nonselective cation channels is an important step for sustained EDHF-mediated response [30, 31].

In contrast to EDHF, endothelium-derived NO production basically correlates with transmembrane Ca<sup>2+</sup> influx, whereas PGI<sub>2</sub> formation is almost entirely dependent from intracellular Ca<sup>2+</sup> pools [33]. Therefore the difference in relative contribution of extra- versus intracellular sources of Ca<sup>2+</sup> for initiation of the synthesis of certain endothelium-derived vasodilator should be of importance. Furthermore, a



**Fig. (2).** Schematic presentation on the current view of the potential mechanisms to endothelium-dependent hyperpolarization. Endothelium-dependent agonists (A) activate EC receptors (R) leading to the entry of extracellular and the release of intracellular  $\text{Ca}^{2+}$  and synthesis of EDHF. Along with synthesis of EDHF the hyperpolarization of ECs occurs, since  $\text{Ca}^{2+}$  activates  $\text{Ca}^{2+}$ -dependent  $\text{K}^{+}$ -channels ( $\text{K}_{\text{Ca}^{2+}}$ ) and induces  $\text{K}^{+}$  efflux. EDHF diffuses to the vascular smooth muscle cells (VSMCs), activates  $\text{K}_{\text{Ca}^{2+}}$ -channels and causes endothelium-dependent hyperpolarization. VSMCs contain voltage-sensitive  $\text{Ca}^{2+}$ -channels ( $\text{vCa}^{2+}$ ) and a drop in membrane potential closes  $\text{vCa}^{2+}$ -channels and induces relaxation. Three main candidates for EDHF nature have been proposed: (1) CYP450 products, (2) potassium ions ( $\text{K}^{+}$ ) and (3)  $\text{H}_2\text{O}_2$ . (1) CYP450 products: An increase in  $\text{Ca}^{2+}$  in EC activates phospholipase  $\text{A}_2$  ( $\text{PLA}_2$ ) known as the rate-limiting enzyme for the liberation of arachidonic acid (AA) from phospholipids (PL). AA is a substrate for CYP450 enzymes (P450). Epoxygenase products of AA, epoxyeicosatrienoic acids (EETs), directly activate  $\text{K}_{\text{Ca}^{2+}}$ -channels in VSMCs and induce relaxation. (2)  $\text{K}^{+}$  *per se*: the opening of EC  $\text{K}_{\text{Ca}^{2+}}$ -channels could result in an increase of extracellular  $\text{K}^{+}$  that could hyperpolarize VSMCs via the activation of the ouabain-sensitive electrogenic  $\text{Na}^{+}/\text{K}^{+}$ , ATPase and inward rectify  $\text{K}^{+}$ -channels ( $\text{K}_{\text{IR}}$ ). (3)  $\text{H}_2\text{O}_2$ : an increase in  $\text{Ca}^{2+}$  in ECs activates enzymes that produce superoxide anions ( $\text{O}_2^-$ ) as a by-product. SOD accelerates the dismutation of  $\text{O}_2^-$  into  $\text{H}_2\text{O}_2$  and molecular oxygen.  $\text{H}_2\text{O}_2$  activates  $\text{K}_{\text{Ca}^{2+}}$ -channels and causes hyperpolarization followed by relaxation. (4) Myoendothelial gap junctions (MEGJ) provide the means by which hyperpolarization of ECs is transferred to VSMCs. MEGJ facilitate the EDHF diffusion from the ECs to VSMCs or may serve as a tie for electrical signal transduction. (Reprinted with permission from Am J Physiol Regul Integr Comp Physiol [32]).

higher endothelial  $[\text{Ca}^{2+}]_i$ ; threshold requirement for EDHF-versus NO-mediated relaxation have been reported recently [34] suggesting that the contribution of EDHF to endothelium-dependent relaxation may depend on environment which predisposes severe alterations in  $[\text{Ca}^{2+}]_i$  concentrations [35].

The nature and distribution of  $\text{K}^{+}$ -channels both within EC and VSMC involved in EDHF-mediated response has yet to be finalized. The importance of several channels have been initially considered, as only the combination of two toxins, i.e. charybdotoxin that block both large ( $\text{BK}_{\text{Ca}}$ ) and intermediate conductance calcium-activated  $\text{K}^{+}$ -channels ( $\text{IK}_{\text{Ca}}$ ), and apamin that inhibits small conductance  $\text{K}^{+}$ -channels ( $\text{SK}_{\text{Ca}}$ ), abolished EDHF-mediated responses [36]. Later, however, utilization of selective inhibitors of  $\text{BK}_{\text{Ca}}$  or  $\text{SK}_{\text{Ca}}$  channels suggested an essential role for  $\text{IK}_{\text{Ca}}$  and  $\text{SK}_{\text{Ca}}$ , but not  $\text{BK}_{\text{Ca}}$ , channels in EDHF-mediated response [37, 38]. Both types of these  $\text{K}^{+}$  channels are localized in EC and are not present in the VSMC [39, 40], indicating an importance of EDH for EDHF-mediated response. In support, apamin

and charybdotoxin prevented EDHF-mediated relaxation when selectively applied to the endothelium but not to superfusion of the vessel [41]. Finally, it can't be excluded that the synthesis and action of EDHF may involve a novel  $\text{K}^{+}$ -channels linked to EC and/or VSMC [37].

The mechanisms responsible for changes in  $\text{K}^{+}$  dynamic on the level of VSMC during EDHF-induced hyperpolarization appears also to be heterogeneous and involve  $\text{BK}_{\text{Ca}}$ , inwardly rectifying  $\text{K}^{+}$ -channels ( $\text{K}_{\text{IR}}$ ) and  $\text{Na}^{+}-\text{K}^{+}$ -ATPase [42, 43]. The variety of mechanisms involved in EDH of VSMC may be due to different nature of EDHF. Indeed, it could be anticipated that EDHF is not a single factor and considerable differences for the nature and cellular targets of EDHF may exist depending on tissue, species and physiological or pathophysiological state.

Up to now the cytochrome P450 products of arachidonic acid [44], potassium ions [45], hydrogen peroxide [46] have been suggested as potential candidates for EDHF. Endothelial cell hyperpolarization may also be transmitted to

smooth muscle cells through myoendothelial gap junctions [47] that are clusters of intercellular channels formed by connexin (Cx) proteins. The major potential candidates for role of EDHF are introduced in (Fig. 2), however for deeper description the readers are advised for recent excellent reviews [11, 28, 48-50].

### **GENDER DIFFERENCES IN EDHF-MEDIATED RESPONSES**

The difference in respect to development of cardiovascular disorders in females and males is well recognized [51, 52], as women at reproductive age appear to be more protected from cardiovascular diseases compared to males [53]. Sex hormones are considered to be of importance but not a sole factor promoting gender-related differences in the development of cardiovascular complications [54, 55]. It has been assumed that one of the mechanisms underlying the protection in females is an enhanced vasodilative capacity of the endothelium [56]. Several studies have demonstrated that basal and endothelium-dependent agonist-induced NO release from endothelium is elevated in arteries from females compared to males [57] and estrogen appeared to be responsible for the gender differences in endothelial NO production [52, 58]. In line, production of cyclooxygenase-dependent products [59] and enhanced EDHF-mediated responses have been shown to be more pertinent to female arteries.

The most convincing evidence for gender-related differences in the relative contribution of EDHF versus NO comes from investigations using animal models. EDHF appeared to be more important in female arteries to confer endothelium-dependent dilatation while NO played a predominant role in arteries from males, as demonstrated in several vascular beds (e.g. mesenteric, tail) from rats [60-62]. These findings suggest that, in the absence of NO contribution, the EDHF can mediate vasodilator responses to agonist or mechanical stress in females rather than in males. Accordingly, if under normal physiological conditions the functional role of EDHF is more significant in females, during the pathological conditions EDHF could compensate the loss of NO in female rather than in male arteries [60].

Recently we have observed (*unpublished data*) that EDHF accounted for gender-related difference in endothelium-dependent relaxation of small femoral arteries from mice, as difference persisted before and after incubation with inhibitors of NO and PGI<sub>2</sub> production. Interestingly, the EDHF-accounted gender-related difference in endothelium-dependent relaxation was a distinguishing feature of small femoral arteries with an internal diameter less than 200µm, since in the bigger arteries the gender related difference in ACh-induced relaxation disappeared further supporting the importance of vessel size in the relative contribution of EDHF.

Moreover, it should be noted that the mechanisms of gender differences in endothelium-dependent relaxation and the relative contribution of EDHF depends on agonist, species and/or anatomic origin of the artery used. It has been demonstrated that gender-specific differences in the contribution of EDHF to endothelium-dependent relaxation in cerebral vasculature differs from that in the periphery. In

the middle cerebral arteries from rats the greater part of endothelium-dependent relaxation in response to ATP accounts for EDHF in males whereas EDHF-mediated relaxation is insignificant in arteries from females [63]. Furthermore, gender *per se* may not necessarily influence EDHF-mediated contribution, since in certain circulations (i.e. resistance arteries from kidney) contribution of EDHF to ACh-induced relaxation is similar in rats of both genders [64].

In humans, however, a gender-related difference in the contribution of EDHF versus NO is less conclusive and has not yet been adequately explored. Although influence of gender on endothelium-dependent relaxation in small arteries from humans has been reported recently, the role of EDHF in mediating gender related differences in endothelium-dependent dilatation remains plausible [65]. The enhanced sensitivity to bradykinin (BK) was observed in omental arteries from females compared to males and inhibition of NO production abolished the gender difference, suggesting the importance of NO, while contribution of EDHF to endothelium-dependent relaxation was up to 90% gender independent. In contrast to females, incubation with inhibitors of NO and PGI<sub>2</sub> production in male subcutaneous arteries increased endothelium-dependent dilatation and, as result, abolished gender-related differences. An enhanced endothelium-dependent relaxation after incubation with inhibitor of NO production in arteries from males could be explained by antagonistic influence of NO on EDHF-mediated responses [66]. However, it is not clear to why antagonistic interplay between NO and EDHF does not emerge in small arteries from females.

Thus, the current data imply that gender difference in endothelium-dependent relaxation is associated not only with the quantitative production of endothelium-derived vasodilators, but also in the relative contribution of NO versus EDHF. In this respect, the investigations on the pooled to one group arteries from women and men, as frequently utilized approach in research of human vascular function *in vitro*, should be considered with caution and not appreciated in the modern physiology.

### **ROLE OF ESTROGEN IN MODULATING OF EDHF-MEDIATED RESPONSES**

The available data demonstrate that pre-menopausal women are protected from cardiovascular disease, however this protection is lost after menopause and significant disease develops in women 10-15 years later than in men. The onset of menopause is often followed by adverse cholesterol changes, increased blood pressure, increased glucose, and increased triglycerides. Although a multitude of changes occur at menopause, it has been proposed that the increase in cardiovascular risk factors is due to the loss of estrogen [67-69].

Thus, female sex hormone-estrogen has been introduced not only as a key factor in gender-related differences in incidence of cardiovascular disease but also in endothelium-dependent maintenance of vascular tone [68], since it has been widely reported that this hormone may enhance endothelium-dependent NO-mediated relaxation in isolated arterial rings, different vascular beds or *in vivo* situation in

females or males, including brachial or resistance arteries [70-73]. More recently, it has been suggested that estrogen modulates not only NO but also production and release of EDHF [74-76].

The evidence that estrogens may affect EDHF-mediated response rather than NO production or release *per se* in response to endothelium-dependent agonists has been attributed as an additional contributory mechanism to cardiovascular protection by hormone replacement therapy [77, 78]. It has been shown that isolated small arteries obtained from animals in estrogen-deficient state induced by ovariectomy are characterized by severely impaired EDHF-mediated hyperpolarization, which is improved by 17 -estradiol replacement therapy [77]. Moreover, ACh induced EDHF-mediated response is greater in mesenteric arteries from male compared to ovariectomized female rats. A possible explanation for the gender difference in EDHF-mediated response has been related to the difference in the concentration of estrogen, since plasma concentration of 17 -estradiol is higher in male rats than in ovariectomized females, probably due to a metabolism of testosterone by aromatase in the adipose tissue [78]. Furthermore, EDHF-mediated response to ACh is suppressed in arteries from diestrus females, although its extent is less severe than in ovariectomized rats, suggesting that even short-term estrogen deficiency could explicitly diminish EDHF-mediated reactivity [76].

The evidence that estrogen favors the contribution of EDHF in the circulation is not pertinent to endothelium dependent agonists-induced responses only. The support exists that estrogen increases EDHF contribution in shear stress mediated dilatation, especially when NO activity is compromised [75]. Flow-induced dilatation of skeletal muscle arterioles in L-NAME-treated male and ovariectomized female rats is solely mediated by prostaglandins whereas estrogen replacement switches that to an EDHF-mediated response, recovering the similar profile of the response observed in NO-deficient arterioles from intact female rats. Furthermore, contribution of EDHF to the acute 17 -estradiol-induced relaxation has been recently reported in the female and male rat coronary arteries. It has been also suggested that EDHF-mediated relaxation in isolated heart represents a constitutive component of 17 -estradiol response as reflected by inhibition of CYP450 metabolite [79].

The mechanism/s behind the beneficial effects of estrogen on EDHF-mediated responses has yet to be finalized. Even if a short-term deficiency in endogenous estrogen may alter EDHF-mediated responses it is possible to suggest that the mechanism/s could be related to functional and reversible alterations in membrane consistence, affecting the ion channels, signal transduction or receptors [76]. As K-channels are the primary target for EDHF-mediated response, the influence of estrogen to enhance the expression of endothelial K-channels strengthens the importance of these channels as an alternative mechanism of estrogen-induced up-regulation of EDHF-mediated responses [19, 80]. It has been also shown that 17 -estradiol may acutely enhance the activity of the Ca<sup>2+</sup>-dependent K-channels in endothelial cells of rabbit aorta [81]

and in human coronary arteries [82]. In addition, chronic treatment with 17 -estradiol prevents impaired EDHF-mediated relaxation in hypercholesterolemic rabbit carotid arteries through activation of both Ca<sup>2+</sup>-dependent and ATP-sensitive K-channels [83] further supporting suggestion about activation of calcium-dependent K-channels by estrogen. The K<sub>IR</sub> channel function could not be excluded due to a recent demonstration that K<sup>+</sup>-induced cerebral vasodilatation *in vivo* is greater in female than in male rats and this vasodilatation is reduced by ovariectomy and restored by 17 -estradiol [84].

The other alternative pathway by which estrogen may induce up-regulation of EDHF-mediated responses in peripheral vasculature is located at the level of cell coupling by gap junctions. Gap junctions are membrane structural proteins that are composed of two hemi channels, which are known as connexons and are formed from six protein subunits or connexins arranged around an aqueous central pore [50]. It has been shown that the number of gap junctions in the myometrial cells depends on hormonal milieu, as the number increases significantly with increasing estrogen and decreasing levels of progesterone [85]. A significant reduction in the expression of the connexin-43 protein, which is present in both EC and VSMC, occurs in mesenteric arteries from ovariectomized rats, while supplementation therapy with 17 -estradiol completely prevents the reduction in the expression of connexin-43 protein in ovariectomized rats to a similar level seen in controls [77]. It might be suggested therefore that estrogen through the certain estrogen receptor (ER) or constantly expression of connexin proteins that may have a profound influence on gap junctional communication and consequently on EDHF-mediated relaxation and could therefore partly explain the gender-related differences.

Recently our group, using ER knockout (ER KO) mice, provided experimental evidence that ER is involved in the regulation of EDHF-mediated responses in small femoral arteries [86], in which gap junctions entirely account for EDHF-induced relaxation. In contrast to wild-type (WT) mice, gender-related difference in ACh-induced dilatation mediated by EDHF disappeared in ER KO animals, indicating that ER has an important impact on gender-related differences in endothelium-dependent relaxation and consequently on EDHF contribution in these responses. Comparison of ACh-induced relaxation between arteries from WT and ER KO females showed similar concentration-response curves to agonist before and after incubation with inhibitors of endothelium-derived factors, whereas arteries from ER KO males were more sensitive to ACh compared to arteries in WT males. These results could be considered as an indirect evidence for down-regulatory role of ER on EDHF-mediated responses in arteries from males. Therefore, it is possible to suggest, that vanished gender-related differences in ACh-induced relaxation in arteries from ER KO mice are attributable to elimination of ER-controlled pathway on EDHF-mediated effects in males, indicating that alterations of ER in peripheral vasculature may have more extensive influence on EDHF-mediated responses in males rather than in females. The physiological compensatory crosstalk between ER and ER in small arteries from females might be more apparent, as

female gender is naturally provided with a more stable sensory system in response to fluctuations in estrogen level during menstrual cycle.

Further details regarding the mechanisms by which estrogen influence EDHF-typed responses in arteries remain to be determined, it is clear that EDHF-mediated dilatation may be altered via mechanisms involving both the classical genomic pathway of steroid hormone activation on protein expression (K-channels and connexins) and non-genomic pathway via effects on  $Ca^{2+}$  activated K-channels.

Further evidence for influence of hormonal environment on EDHF-mediated responses is provided in the next chapter, where an increase role for EDHF in the endothelium-dependent relaxation in pregnancy is introduced.

### **EDHF-MEDIATED RESPONSES IN NORMAL PREGNANCY AND PREECLAMPSIA**

Maternal cardiovascular adaptation to pregnancy is associated with decreased peripheral vascular resistance that plays an important role for blood pressure reduction despite an increase in plasma volume and cardiac output. The vascular adaptation to pregnancy depends on enhanced endothelium-dependent dilatation [87, 88]. Preeclampsia is an endothelial cell disorder and an increased vascular resistance and blood pressure during this pregnancy-related disorder is due to endothelial dysfunction and severe alterations in endothelium-dependent relaxation [89, 90]. The mechanisms underlying endothelium-dependent relaxation in normal and compromised pregnancy has attracted considerable interest due to the rationale to design novel therapeutic models to prevent or relief the symptoms of endothelial dysfunction.

It is now generally accepted that NO plays an important role as a systemic vasodilator in pregnancy [91]. More recently, a role for EDHF in the increased endothelium-dependent relaxation in pregnancy has also been introduced [92] and several studies aimed to clarify nature and contribution of EDHF-type responses to endothelium-dependent relaxation in pregnancy. It has been shown, that EDHF played an important role in the enhancement of ACh-induced relaxation in the aorta and small mesenteric arteries from pregnant rats [93, 94]. If experimental evidence in animal pregnancy is more explicit, the anticipated increased contribution of EDHF in human pregnancy is less conclusive due to a lack of proper comparison with arteries from non-pregnant women [32, 95, 96]. A significant inter-individual heterogeneity in the relative contribution of NO versus EDHF to endothelium-dependent relaxation particularly in human arteries may also affect elucidation. In this respect, it is important to stress that contribution of EDHF to increased endothelium-dependent relaxation in human arteries may also depend from vascular bed and agonist used, suggesting, in general, a complexity of EDHF-mediated response in human pregnancy.

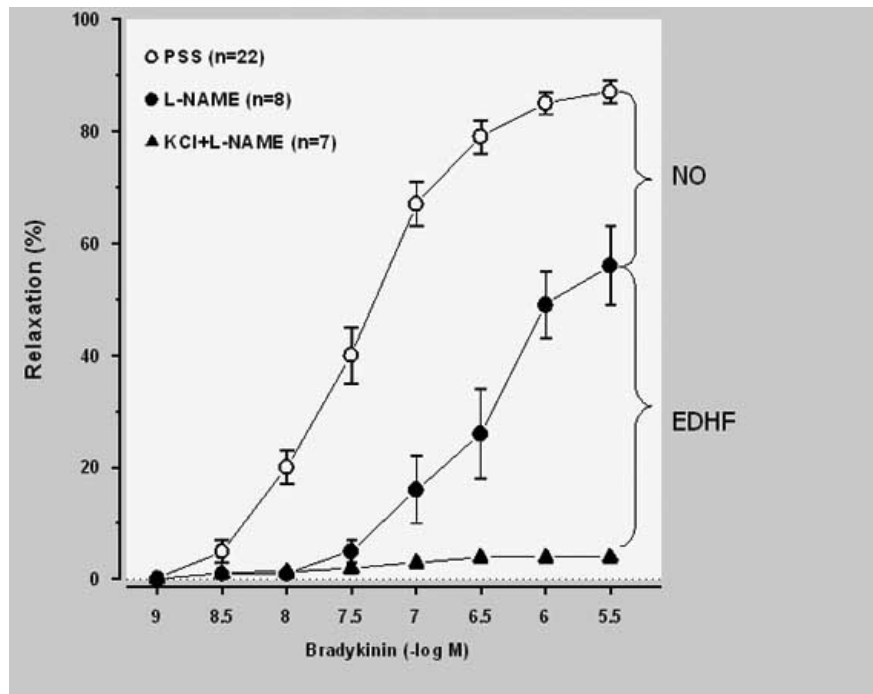
Small subcutaneous arteries from pregnant women demonstrate a residual bradykinin (BK)-induced relaxation after incubation with inhibitors of NO and  $PGI_2$  production to be significantly higher, as confirmed by differences in  $EC_{50}$  values, compared to that in non-pregnant women [95]. In contrast, Ang et al failed to show any difference in

contribution of EDHF to carbachol-induced relaxation in the same arteries from pregnant and non-pregnant women [97]. An endothelium-dependent relaxation in response to both ACh and BK is completely accounted for EDHF in small omental arteries, however no difference exists in EDHF-typed contribution between pregnant and non-pregnant women [96]. In myometrial arteries obtained from normal pregnant women, the absence of NO has been shown to be compensated by EDHF-mediated response in response to BK, an effect not apparent in arteries from non-pregnant women [98]. An equal contribution of EDHF to endothelium-dependent relaxation has been demonstrated in mesenteric arteries from pregnant and non-pregnant mice, whereas a decreased role of EDHF was relevant to uterine arteries in mice pregnancy [99].

The mechanism/s responsible for up-regulation of EDHF-mediated responses remains far from clear. One feasible suggestion could be related to the increased levels of circulating estrogen in pregnant versus non-pregnant state. Moreover, the diversity in contribution of EDHF to endothelium-dependent relaxation in pregnancy in respect to the type of vessels used could be related to the different nature of EDHF in specific vascular bed. In addition, implication could be made that the mechanism of EDHF-mediated response may differ in certain vascular bed in pregnant versus non-pregnant state. Up to date, however, there have been few attempts to clarify these issues.

Pascoal and Umans have showed that EDHF-mediated response to BK in omental arteries is abolished after incubation with inhibitor of non-selective K-channels in non-pregnant but not in pregnant women, suggesting different nature of EDHF [96]. Recently, the nature of EDHF in human pregnancy has been explored in small subcutaneous [32] and myometrial arteries [98]. It has been shown that BK-induced relaxation is almost equally mediated by NO and EDHF in subcutaneous arteries from normal pregnant women (Fig. 3) and CYP450 epoxygenase metabolites of AA or  $H_2O_2$  did not account for EDHF-mediated response, while gap junctions were involved in the EDHF-mediated responses (Fig. 4) [32]. In this study the mechanism of EDHF-mediated response in arteries from non-pregnant women have not been investigated, but comparison with data from other studies suggests that the nature and role of EDHF in subcutaneous circulation might differ in pregnant versus non-pregnant state. CYP450-dependent products of AA metabolism in EDHF-mediated responses have been suggested to be of importance in subcutaneous arteries from healthy non-pregnant volunteers [100], but not from patients with cancer or cardiovascular disease [101], suggesting that mechanism of EDHF-mediated responses might also differ in healthy versus diseased condition.

In support to our findings in subcutaneous arteries, Kenny et al demonstrated that several distinct agents interfering with gap junctions inhibited EDHF-mediated responses to BK in isolated small myometrial arteries from normal pregnant women [102]. This could suggest that gap junctions are of importance to EDHF-type responses in several vascular beds in human pregnancy. This is amplified by studies in pregnant animals, where the contribution of EDHF to ACh-induced relaxation was significantly



**Fig. (3).** NO and EDHF almost equally account for BK-induced relaxation in small subcutaneous arteries isolated from normal pregnant women, since incubation with inhibitors of NO and PGI<sub>2</sub> production (L-NAME) reduced relaxation up to 50% from level of previous relaxation in physiological salt solution (PSS). Incubation with L-NAME in K<sup>+</sup>-modified solution abolished BK-induced responses suggesting that residual dilatation accounted for EDHF. (Adopted with permission from Am J Physiol Regul Integr Comp Physiol [32]).

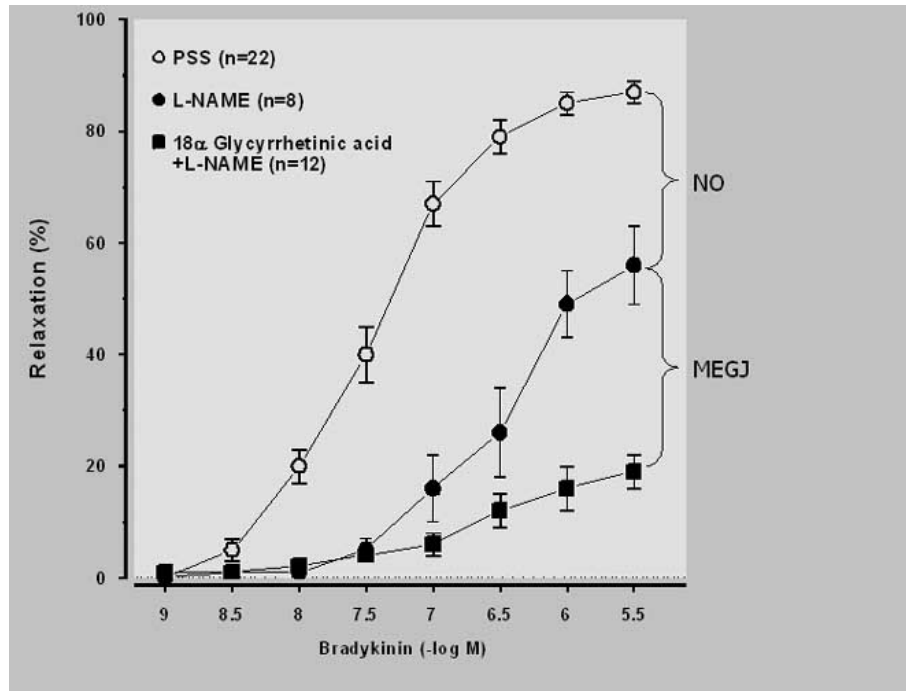
enhanced in aorta due to an increased contribution of gap junction communication, which could presumably facilitate EDHF diffusion from the endothelium to SMC [94]. This was accompanied by the increased expression of connexin-43 mRNA not only in the thoracic aorta but also mesenteric and uterine arteries [94].

All experimental evidence so far strengthens the hypothesis that EDHF is equally important as NO oxide to the enhanced vasodilative capacity and that gap junctional communications confer EDHF-mediated responses in pregnancy at least in subcutaneous and myometrial vascular beds, where a substantial increase in blood flow occurs. It remains of importance, however, to identify the nature of EDHF and to clarify its contribution to endothelium-dependent relaxation in preeclampsia, since this disorder is characterized by a generalized endothelial dysfunction accompanied by alterations in endothelium-dependent dilatation and increased vascular resistance [89, 90].

In several studies the relative contribution of EDHF versus NO in arteries from preeclamptic women has been investigated. Pascoal et al have demonstrated differences in endothelium-dependent relaxation in omental arteries from women with preeclampsia and normal pregnant depending on the agonist used: the response to ACh, but not to BK, was entirely abolished, suggesting a defect of muscarinic signal transduction rather than changes in endothelium-derived relaxing factor *per se* [103]. It should be stressed that in this study endothelium-dependent relaxation to BK was NO/prostanoid-independent, but entirely EDHF-mediated. In

contrast, Suzuki et al using strips of omental arteries from normal and preeclamptic women demonstrated almost equal contribution of NO and EDHF to BK and substance P-induced relaxation, and implied that the role of NO, but not EDHF, is impaired in preeclampsia [104]. A significant reduction in BK-induced PGI<sub>2</sub>-mediated hyperpolarization (but not EDHF-mediated one) in omental arteries from preeclamptic women has been demonstrated by the same group later, suggesting that the function of NO and PGI<sub>2</sub> is down-regulated, whereas EDHF function remains normal. [105]. Thus, the discrepancy in results obtained in omental arteries in preeclampsia between different research groups deserves consideration and requires further investigation.

Much attention has been paid to endothelium-dependent relaxation in myometrial arteries from women with preeclampsia. Using a wire myography technique Ashworth et al showed a significant reduction of endothelium-dependent relaxation to BK in preeclampsia [106]. More recently, however, utilizing pressure myography technique Kenny et al failed reproduce the results, since concentration-response curves to BK were identical in arteries from normal pregnant and preeclamptic women [98]. Furthermore, the last study clarified the mechanism of endothelium-dependent responses to BK and demonstrated that an illusive contribution of EDHF to endothelium-dependent relaxation in preeclampsia is similar to that in arteries from non-pregnant women. In normal pregnancy, when NO production is inhibited, myometrial arteries developed an EDHF-mediated vasodilator response to BK that is comparable to



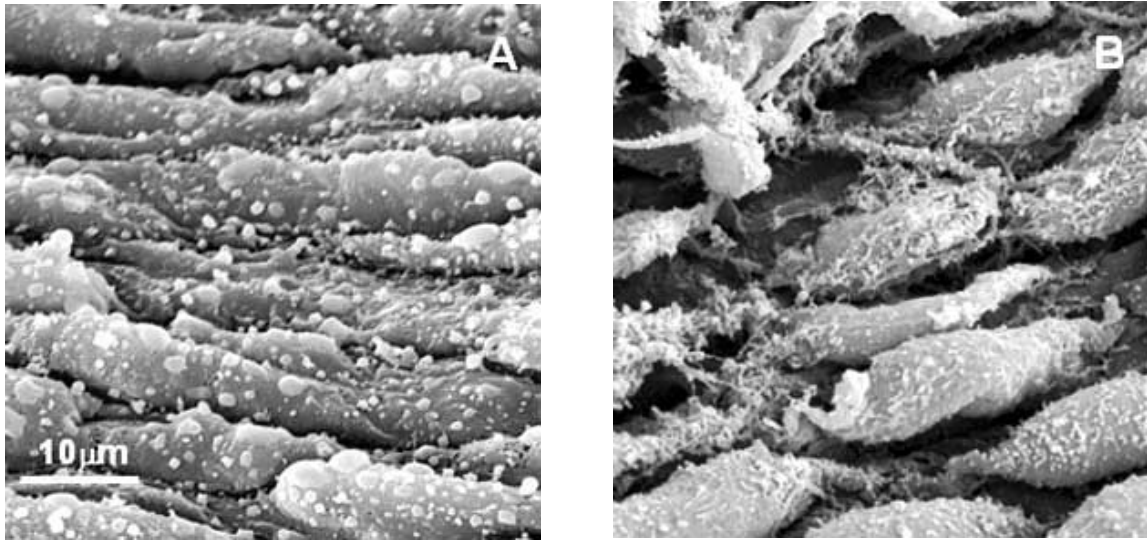
**Fig. (4).** Myoendothelial gap junctions (MEGJ) are involved in EDHF-mediated responses in small subcutaneous arteries isolated from normal pregnant women, since incubation with 18-glycyrrhetic acid (inhibitor of MEGJ) significantly reduced EDHF-mediated component of relaxation. (Adopted with permission from *Am J Physiol Regul Integr Comp Physiol* [32]).

that before incubation with inhibitor of NO. It has been suggested that EDHF-dependent compensatory mechanism represents a vascular adaptation of myometrial arteries to normal pregnancy, which is absent in preeclampsia, contributing to the clinical features of the disorder [98].

Endothelium-dependent relaxation in small subcutaneous arteries isolated from women with preeclampsia has been also investigated and, in general, suggested that different mechanism/s responsible for the reduction of endothelium-dependent dilatation might depend on agonist used. The degree of reduction in ACh-induced relaxation was modest and accounted for the defect in PGI<sub>2</sub>-mediated component of the response [107]. In contrast, the relaxation response to BK was significantly reduced before and after incubation with inhibitor of NO production suggesting involvement of NO- and EDHF-mediated components [95]. The authors, however, suggested that blunted BK-induced relaxation in preeclampsia accounts for reduction in NO production, because inhibitor of NO-synthase appears to reduce, but not abolish, the difference in pEC<sub>50</sub> values between arteries from women with normal versus preeclamptic pregnancy. On the other hand, the same level of reduction in maximum relaxation to BK before and after incubation with inhibitor of NO production (i.e. 12% versus 15%) may indirectly support the reduced contribution of EDHF [95].

So far, there has been not enough evidence to suggest that compromised NO-mediated response could alone completely explain endothelial dysfunction in subcutaneous arteries in preeclampsia and it is possible that changes in EDHF or PGI<sub>2</sub> production might be involved depending on

the agonist used. Moreover, it can't be excluded that depending on vascular bed studied EDHF may account for one mechanism in normal pregnancy, whereas in preeclampsia the nature and contribution of EDHF varies depending on the heterogeneity in the pathophysiology behind preeclampsia or severity of the disease. In support to this we have compared the relative contribution of NO versus EDHF to BK-induced relaxation in subcutaneous arteries and found that endothelium-dependent relaxation is reduced to the same extent before and after incubation with inhibitors of NO and PGI<sub>2</sub> production in arteries from preeclampsia versus normal pregnant women. In contrast to normal pregnancy, gap junctions did not entirely accounted as a single mechanism of EDHF-mediated response in arteries from women with preeclampsia and it was combined either with CYP450 metabolites or hydrogen peroxide. In line with the function evidence of endothelial dysfunction during preeclampsia, morphological alterations of endothelial cell layer are well established and found in kidney's vasculature [108], myometrial [109, 110] and subcutaneous arteries [111]. In arteries from normal pregnant women, a continuous layer of elongated and tightly connected with each other EC is providing a perfect environment for gap junction communications, whereas in arteries from women with preeclampsia, many of endothelial cells appear to be considerably increased in size, edematous, separate and detached from neighboring cells that makes intercellular communications quite difficult or even impossible (Fig. 5). Thus, it is possible that in preeclampsia EDHF-mediated responses in subcutaneous and even in



**Fig. (5).** Scanning electron microscopy pictures of endothelial cell layer. In subcutaneous arteries from normal pregnant women (A), endothelial cells are tightly connected with each other providing a perfect environment for gap junction communications. In arteries from women with preeclampsia (B), endothelial cells appear to be increased in size, edematous, more separate and isolated from neighboring cells and basal membrane that makes intercellular communications quite difficult or even impossible.

myometrial arteries should be damping out or accounted by another pathway/s rather than gap junctions.

#### CONCLUSIONS AND FUTURE PERSPECTIVE

Summarizing the available evidence so far we suggest that EDHF, rather than NO, is of importance for the regulation of resistance artery function in females. It has a significant impact on the control of organ blood flow to target organs and blood pressure, which are the two major elements targeted in the majority of cardiovascular complications. Contribution of EDHF to the endothelium-dependent dilatation in small female arteries might be altered due to required situation in order to preserve the vasodilative capacity of the endothelium. Moreover, EDHF-mediated response is particularly sensitive to changes in the hormonal environment, as several pathways involved in the EDHF signaling seem to be susceptible for estrogenic benefit. Considering the evidence about endothelial dysfunction as a pathogenetic hallmark of the diseases peculiar to female gender, including preeclampsia and increased risk to develop cardiovascular complications after menopause, the knowledge about EDHF should be of interest for both clinicians and scientists.

Several approaches to improve EDHF-mediated response are currently of interest, although the mechanisms responsible for this are far from clear. The improved EDHF-mediated responses have been found after treatment with antioxidants [112], statins [113] and calcium dobesilate [114] in arteries from patients with hypertension and hypercholesterolemia. Chronic treatment with selective estrogen modulators and phytoestrogens might be considered as an alternative to improve EDHF-mediated response in conditions associated with estrogen deficiency [16]. Folate was introduced as candidate to improve endothelial function

in patients with hypercholesterolemia [115], hyperhomocysteinaemia [116], coronary artery disease [117], diabetes [118] or in women after menopause [119]. Moreover, *in vitro* experiments have shown that folate may improve EDHF-mediated response independent of its well-known role on homocysteine metabolism or anti-oxidative properties [118]. It is of interest therefore whether folate could improve endothelial function in arteries from women with preeclampsia and after menopause.

The heterogeneity in contribution of EDHF to endothelium-dependent relaxation and its illusive nature provides an intriguing challenge for further investigations. The understanding of nature and role that EDHF plays in endothelium-dependent dilatation in peripheral circulation in normal and diseased state will hopefully enable us to elaborate the development of new therapeutic approaches to improve endothelial function in preeclampsia and cardiovascular diseases in respect to gender and/or after menopause.

#### ACKNOWLEDGEMENTS

Supported by grants from Harald Jeansson and The Harald & Greta Jeansson foundation, Swedish Society of Medicine, Swedish Heart and Lung foundation, Ageing foundation from Karolinska Institutet.

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