EDHF mediates flow-induced dilation in skeletal muscle arterioles of female eNOS-KO mice

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Departments of ¹Physiology, ²Pharmacology, and ³Pathology, New York Medical College, Valhalla, New York 10595; ⁴Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas 75235; and ⁵Division of Hypertension and Vascular Research, Henry Ford Hospital, Detroit, Michigan 48202

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Huang, An, Dong Sun, Mairead A. Carroll, Houli Jiang, Carolyn J. Smith, Joseph A. Connettea, John R. Falck, Edward G. Shesely, Akos Koller, and Gabor Kaley. EDHF mediates flow-induced dilation in skeletal muscle arterioles of female eNOS-KO mice. Am J Physiol Heart Circ Physiol 280: H2462–H2469, 2001.—Vasodilation to increases in flow was studied in isolated gracilis muscle arterioles of female endothelial nitric oxide synthase (eNOS)-knockout (KO) and female wild-type (WT) mice. Dilation to flow (0–10 μm/s) was similar in the two groups, yet calculated wall shear stress was significantly greater in arterioles of eNOS-KO than in arterioles of WT mice. Indomethacin, which inhibited flow-induced dilation in vessels of WT mice by ~40%, did not affect the responses of eNOS-KO mice, whereas miconazole and 6-(2-proparglyoxyphenyl)hexanoic acid (PPOH) abolished the responses. Basal release of epoxyeicosatrienoic acids from arterioles was inhibited by PPOH. Iberiotoxin eliminated flow-induced dilation in arterioles of eNOS-KO mice but had no effect on arterioles of WT mice. In WT mice, neither Nω-nitro-ω-arginine methyl ester nor miconazole alone affected flow-induced dilation. Combination of both inhibitors inhibited the responses by ~50%. 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) alone inhibited flow-induced dilation by ~49%. ODQ + indomethacin eliminated the responses. Thus, in arterioles of female WT mice, nitric oxide and prostaglandins mediate flow-induced dilation. When eNOS is inhibited, endothelium-derived hyperpolarizing factor substitutes for nitric oxide. In female eNOS-KO mice, metabolites of cytochrome P-450, via activation of long-conductance Ca2+-activated K+ channels of smooth muscle, mediate entirely the arteriolar dilation to flow.

nitric oxide; prostaglandins; hyperpolarizing factor; cytochrome P-450 metabolites; potassium channels

NITRIC OXIDE (NO), prostaglandins, and endothelium-derived hyperpolarizing factor (EDHF) represent three major endothelial factors involved in the local regulation of vascular tone (4, 14). Attributing specific effects to each of these factors is hampered by the fact that all three mediators may be simultaneously synthesized in and/or released from endothelial cells in response to a single stimulus, e.g., shear stress (21) or vasoactive agents (16). The mechanisms by which release of NO or prostaglandins is activated by alterations in wall shear stress (WSS) during changes in blood flow have been extensively studied (4, 21). The release of EDHF in response to vasoactive agents, as well as to hemodynamic stimuli such as pulsatile stretch, has also been demonstrated in a variety of vascular beds (1, 16, 28). However, little experimental evidence has been provided to suggest that the release of EDHF can be stimulated by shear stress, a physiologically relevant stimulus in vivo.

The term EDHF may prove to represent a group of different factors, since not all EDHF-mediated vascular responses display the same behavior or the same sensitivity to pharmacological agents (6, 9, 11, 22, 33). In coronary, cerebral, renal, and skeletal muscle circulations, EDHF has been characterized as a cytochrome P-450 (CYP) epoxyxygenase metabolite(s) of arachidonic acid [epoxyeicosatrienoic acids (EETs)], thought to hyperpolarize vascular smooth muscle by opening K+ channels (1, 10, 12, 16).

Our previous studies demonstrated that, whereas endothelial NO and prostaglandins mediate flow-dependent dilation in gracilis muscle arterioles in male wild-type (WT) mice, an upregulation of prostaglandin synthesis is solely responsible for the maintenance of this response in male endothelial NO synthase (eNOS)-knockout (KO) mice (32). Estrogen was shown to potentiate shear stress-dependent dilation of arterioles, suggesting that the NO-stimulating effect of the hormone is one of the key mechanisms that underlies the gender difference in the regulation of arteriolar tone (17). In addition, estrogen increases coronary blood flow and improves vascular dysfunction by opening Ca2+-dependent K+ (KCa) channels (26) and by potentiating EDHF-mediated, agonist-induced vasorelax-
ation (23). The question, therefore, arose as to whether there is a gender difference in the compensatory mechanisms that accounts for the preservation of flow-induced dilation in eNOS-KO mice. We hypothesized that, because of a targeted disruption of the gene encoding eNOS, endothelial mediators other than NO maintain a close-to-normal dilation to flow/shear stress in skeletal muscle arterioles of female mice. Thus we aimed to elucidate the nature of the endothelial mediators responsible for flow-induced dilation in gracilis muscle arterioles of female eNOS-KO and WT mice and then to identify the gender difference, if any, in the endothelial regulatory mechanisms.

METHODS

Animals. Heterozygous eNOS (+/−) mice, originally developed by Shesely et al. (30), were interbred to generate eNOS-WT (+/+ ) and homozygous mutant (−/−) mice. Mice were genotyped by Southern analysis of DNA as described previously (30). All protocols were approved by the Institutional Animal Care and Use Committee of New York Medical College and conform to the National Institutes of Health and American Physiological Society guidelines for the use and care of laboratory animals. eNOS-KO and WT mice were bred in the Department of Comparative Medicine at New York Medical College.

Experimental setup. Experiments were conducted on isolated gracilis muscle arterioles of female eNOS-KO and WT mice. The mice were killed and the vessels were dissected and isolated as described previously (16, 32).

Experimental procedures. Changes in diameter of arterioles in response to increases in flow were studied at 80 mmHg of perfusion pressure. Perfusate flow was increased from 0 to 10 μl/min in 2 μl/min steps.

In the first series of experiments, the role of prostaglandins in flow-induced dilation was assessed by using indomethacin (Indo, 10−5 M), an inhibitor of cyclooxygenase, after control flow-diameter curves were obtained.

In the second series of experiments, the role and interaction of metabolites of CYP and eNOS in flow-induced dilatation were assessed by using miconazole (MCZ, 2 × 10−6 M) and 6-(2-proparglyoxyphenyl)hexanoic acid (PPOH, 10−6 M), an inhibitor of CYP epoxygenase (14, 34), and Nω-nitro-l-arginine methyl ester (l-NAME, 10−4 M), an inhibitor of NOS. After control experiments, MCZ or l-NAME was administered first for 30 min before the experiments were repeated. Flow-induced responses were then studied once more in the additional presence of l-NAME or MCZ. In a separate group of experiments, the effect of metabolites of CYP on flow-induced dilation in arterioles of eNOS-KO mice was confirmed further by using PPOH, though to be a more specific inhibitor of CYP epoxygenase (34).

In the third series of experiments, the contribution of EDHF to flow-induced responses was evaluated by performing the experiments before and after extraluminal administration of ibetoxin (IBTX, 2 × 10−8 M), a blocker of large-conductance KCa channels.

In the fourth series of experiments, the participation of the l-arginine-NO-cGMP pathway in the flow-induced responses of arterioles from WT mice was tested by using l-NAME or 1H-[1,2,4]oxadiazolo[4,3-a]quinazolin-1-one (ODQ, 3 × 10−5 M), an inhibitor of guanylate cyclase. The inhibitors were administered alone or simultaneously with MCZ or Indo.

Passive diameter. At the conclusion of each experiment, the suffusion solution was changed to a Ca2+-free solution containing 1 mM EGTA. Vessels were incubated for 10 min to reach maximal diameter. Passive diameters were assessed by using miconazole (MCZ, 2 × 10−6 M), an inhibitor of CYP epoxygenase (34), and dibromododecynyl-methylsulfimide (3 × 10−5 M), an inhibitor of ω-hydroxylase, at 37°C for 1 h. The vessel and media eicosanoids were extracted after addition of 4.5 ng of a mixture of D8-EETs (8,9-, 11,12-, and 14,15-EET) as internal standards.

Quantitation of EETs. To quantify the basal release of EETs by arterioles (control group), as well as the specific inhibitory effect of PPOH (3 × 10−5 M) on this release (PPOH group), arterioles isolated from three WT mice from each group (13 μg protein/ml) were incubated in the presence of NADPH (1 M), Indo (3 × 10−5 M), and dibromododecynyl-methylsulfimide (3 × 10−5 M), an inhibitor of ω-hydroxylase, at 37°C for 1 h. The vessel and media eicosanoids were extracted after addition of 4.5 ng of a mixture of D8-EETs (8,9-, 11,12-, and 14,15-EET) as internal standards.

Purification of EETs. The EETs were purified using reverse-phase HPLC and derivatized and quantitated by negative chemical ionization gas chromatography (GC)-mass spectroscopy (MS) as described previously (7). Briefly, the samples were extracted twice with two volumes of acidified ethyl acetate (pH 4.0) and evaporated to dryness. The samples were purified by reverse-phase HPLC on a C18 μm column (30 × 4.6 mm) using a linear gradient from acetonitrile-water-acetic acid (62.5:37.5:0.05%) to acetonitrile (100%) over 20 min at a flow rate of 1 ml/min. Fractions containing EETs were collected on the basis of the elution profile of standards monitored by ultraviolet absorbance (205 nm). The fractions were evaporated to dryness and derivatized for GC-MS analysis.

Derivatization and MS analyses. Pentafluorobenzyl esters were prepared by the addition of α-bromo-2,3,4,5,6-pentafluorotoluene (pentafluorobenzylbromide, 5 μl; Aldrich) and N,N-diisopropylethylamine (5 μl; Aldrich) to a sample dissolved in acetonitrile (100 μl), and the derivatization was continued at room temperature for 30 min. Samples were dissolved in isooctane, and 1-μl aliquots were injected into a GC (model HP-5890, Hewlett-Packard) column (15.0 m, 0.25 mm ID, 0.25 μm film thickness; DB-1, Supelco) using a temperature program ranging from 150 to 300°C at a rate of 10°C/min. Methane was used as a reagent gas at a flow resulting in a source pressure of 1.3 Torr, and the MS (model 5989A, Hewlett-Packard) was operated in electron capture chemical ionization mode. The endogenous EETs were identified (ion mass-to-charge ratio = 319) by comparison of GC retention times with authentic D8-EETs (mass-to-charge ratio = 327) standards and quantitated by calculating the ratio of abundance.

Chemicals. All chemicals were obtained from Sigma (St. Louis, MO). PPOH was dissolved in ethanol at 10−2 M and further diluted with physiological saline solution.

Calculations and statistics. Changes in diameter in response to increases in flow/shear stress in each vessel were normalized by its passive diameter. WSS at each flow rate and that required to cause 50% of maximal dilation (WSS0) were calculated (32). Statistical significance was calculated by repeated-measures ANOVA followed by the Tukey-Kramer multiple-comparison test. Values are means ± SE. When two or more vessels were studied from one animal, the responses were averaged. The GC-MS data were analyzed by a group t-test on logarithmic transformation of the data. Significance level was taken at P < 0.05.

RESULTS

The characteristics of arterioles isolated from gracilis muscle of female WT (N = 28) and eNOS-KO (N = 20) mice are summarized in Table 1. Active and passive diameters were significantly smaller in arterioles of eNOS-KO mice than in arterioles of WT mice, whereas the basal arteriolar tone, expressed as percentage of
passive diameter, was not significantly different in vessels of the two groups of mice.

Increasing flow from 0 to 10 μl/min elicited significant increases in diameter of arterioles from WT and eNOS-KO mice. The maximal changes in diameter were not significantly different (31.6 ± 1.1 and 29.9 ± 1.1 μm at 10 μl/min, respectively). The dilations of arterioles from eNOS-KO and WT mice were nearly identical, as shown in Fig. 1A, where the normalized diameter as a function of perfusate flow is depicted.

Fig. 1. Normalized diameter of gracilis muscle arterioles (A) and calculated shear stress (B) as a function of perfusate flow in female wild-type (WT; 54 vessels from 28 animals) and endothelial nitric oxide synthase (eNOS)-knockout (KO; 32 vessels from 17 animals) mice. *Significant difference between the two curves (by ANOVA).

Fig. 2. Normalized diameter of gracilis muscle arterioles of female WT (12 vessels from 6 animals) mice as a function of perfusate flow in control conditions and in the presence of indomethacin (Indo, 10^-5 M). PD, passive diameter. *Significant difference from control.

Fig. 8 shows that the basal release of EETs from gracilis muscle arterioles of mice was substantial, and PPOH specifically inhibited this release by ~40%.

Table 1. Characteristics of gracilis muscle arterioles of mice

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>WT (n = 28)</th>
<th>eNOS-KO (n = 20)</th>
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<tbody>
<tr>
<td>Age, wk</td>
<td>31.0 ± 1.3</td>
<td>30.0 ± 3.3</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>25.8 ± 1.0</td>
<td>24.1 ± 1.0</td>
</tr>
<tr>
<td>Diameter, μm Basal</td>
<td>82.6 ± 1.4</td>
<td>73.4 ± 2.4*</td>
</tr>
<tr>
<td></td>
<td>137.2 ± 1.9</td>
<td>122.0 ± 3.3*</td>
</tr>
<tr>
<td>Basal tone, % of passive diameter</td>
<td>60.2 ± 0.6</td>
<td>60.0 ± 0.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of mice. WT, wild-type; eNOS-KO, endothelial nitric oxide synthase-knockout. *Significant difference from WT mice.

and eNOS-KO mice (summarized in Figs. 2–5 and Figs. 6 and 7, respectively) were investigated.

The role of prostaglandins in the mediation of flow-induced dilation in vessels of WT mice is shown in Fig. 2. Indo, which did not affect basal tone in arterioles of either strain of mouse, significantly inhibited flow-induced dilation by ~40% (P < 0.05) in arterioles of WT mice. In a separate group of experiments (Fig. 3), the roles of NO and CYP metabolites in the mediation of flow-induced dilations in WT arterioles were assessed by using l-NAME and MCZ, respectively. Neither l-NAME alone nor MCZ alone affected flow-induced dilations and the basal tone of vessels. Combination of both inhibitors, however, significantly inhibited the responses (by ~50%; Fig. 3). The remaining portion of the dilator responses was essentially eliminated by additional administration of Indo (Fig. 4). ODQ, an inhibitor of guanylate cyclase, was then used to confirm the role of the NO-cGMP pathway in the mediation of flow-induced dilations in control conditions. Figure 5A shows that, unlike l-NAME, ODQ alone, while having no effect on basal tone, significantly inhibited flow-induced dilation by (~49%), which was not affected further by MCZ. However, the residual portion of the responses in the presence of ODQ was abolished by additional administration of Indo. IBTX, a blocker of large-conductance K_Ca channels, a target of CYP metabolites/EDHF in smooth muscle (16), did not affect the response in control conditions (Fig. 5B).

In arterioles of eNOS-KO mice, flow-induced dilation was independent of prostaglandins, since Indo had no effect on the response. The response, however, was eliminated by MCZ or PPOH (Fig. 6), suggesting an involvement of the CYP pathway in the mediation of the responses to flow. When arterioles from eNOS-KO mice were treated with IBTX, flow-induced dilation was abolished (Fig. 7).

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DISCUSSION

The new findings of the present study suggest that in female eNOS-KO mice the endothelium-dependent dilation of isolated skeletal muscle arterioles to flow/shear stress is close to normal and is likely to be mediated by EETs, which could be viewed as EDHF. In contrast, in arterioles of female WT mice, corelease of NO and prostaglandins is responsible for the mediation of flow-induced dilation. Also, in vessels of WT mice, when eNOS is inhibited by the acute administration of l-NAME, EETs substitute for NO in the mediation of flow-dependent dilation.

Our previous study demonstrated that EDHF is responsible for dilation to ACh of skeletal muscle arterioles of male eNOS-KO mice, while NO seems to account almost entirely for the dilation in vessels of corresponding WT mice (16). A similar phenomenon has also been observed regarding the participation of NO and EDHF in agonist-induced dilations of porcine epicardial arteries and dog coronary arterioles before and after acute inhibition of NO synthesis (20, 25). Also, the hyperpolarization of coronary smooth muscle elicited by ACh can be inhibited by exogenously applied endothelial prostanoids (36). On the basis of these findings, it seems plausible that the significance of the contribution of EDHF to the regulation of vascular tone in physiological conditions is compromised by the presence of NO and prostaglandins, since EDHF-mediated responses appear only after inhibition of eNOS and/or cyclooxygenase (2, 3). In this context, the experimental model of mice deficient in the gene for eNOS becomes a valuable probe in the investigation of the interactions among these enzyme systems. Indeed, we previously found that, in male eNOS-KO mice, flow/shear stress-induced dilation of skeletal muscle arterioles is mediated exclusively by prostaglandins, instead of by both NO and prostaglandins in control (WT) mice (32).

The effect of estrogen on enhancing the release of endothelial mediators, especially NO, has been well documented (18). On the other hand, it was also reported that pregnancy induces a significant increase in the activity of CYP (29) and causes an upregulation of gap junction protein expression, which may well be responsible for the augmented ACh-induced dilation (8). The importance of gap junctional communication in the mediation of EDHF-induced vasodilation, especially in the microvessels, has attracted considerable attention (24). Moreover, it was also reported that estrogen favors the contribution of EDHF over NO in the mediation of agonist-induced vasodilation (13, 23).

Given that there is a negative interaction among the activity of endothelial mediators and that female hormones favor EDHF-mediated responses, it was plausible to speculate that in female eNOS-KO mice the synthesis/activation of EDHF is upregulated in response to shear stress. To test this hypothesis, flow-induced dilation and the role of endothelial factors mediating this response were investigated in gracilis muscle ar-

Fig. 3. Normalized diameter of gracilis muscle arterioles of female WT mice as a function of perfusate flow in the control condition, in the presence of Nω-nitro-L-arginine methyl ester (l-NAME, 10⁻⁴ M) and l-NAME + miconazole (MCZ, 2 x 10⁻⁶ M; A; 9 vessels from 5 animals), or in the presence of MCZ and MCZ + l-NAME (B; 8 vessels from 5 animals). *Significant difference from control and l-NAME or from control and MCZ.

Fig. 4. Normalized diameter of gracilis muscle arterioles of female WT (16 vessels from 8 animals) mice as a function of perfusate flow in the control condition, in the presence of l-NAME + MCZ, and in the presence of l-NAME + MCZ + Indo. *Significant difference from control and from l-NAME + MCZ, and in the presence of l-NAME + MCZ + Indo.
terioles of female eNOS-KO and WT mice, the type of vessels that we previously studied in male littermates (32).

The average age and body weight of the two strains of mice were comparable; however, the active and passive diameters of arterioles of eNOS-KO mice were significantly smaller than those of WT mice. As a result, the basal tone of vessels in the two strains of mice was comparable, indicating a similar responsiveness to intraluminal pressure.

Flow-induced dilation in arterioles of WT and eNOS-KO mice. In response to increases in perfusate flow, arterioles of both strains of mice exhibited substantial dilations that were similar in magnitude, suggesting that, despite the absence of NO synthesis in endothelium of arterioles from eNOS-KO mice, dilation to increases in flow is essentially preserved. However, the effect of the lack of eNOS was revealed by a significant leftward shift of the flow-shear stress curve in eNOS-KO arterioles (Fig. 1B), indicating that these vessels require a greater shear stress than those of WT mice to achieve a dilation of similar magnitude. A greater WSS50 in vessels of eNOS-KO mice is also indicative of a reduced endothelial sensitivity to shear stress.

Endothelial mediators contributing to flow-induced dilation in arterioles of female WT mice. Flow-induced dilation in arterioles of WT female mice is mediated, in part, by endothelial prostaglandins, as manifested by the significant inhibition of the response by Indo administered in the control condition (Fig. 2). Interestingly, L-NAME did not affect the response, suggesting that in control conditions NO may not be involved in the generation of flow-induced dilation. Additional administration of the CYP blocker MCZ did, however, result in a ~50% inhibition of the response (Fig. 3A). To evaluate the direct role of CYP metabolites in the mediation of the responses, MCZ was then given in the control condition. Similar to L-NAME, MCZ alone did not affect the responses, unless it was administered...
simultaneously with L-NAME (Fig. 3B). We interpret these findings to mean that there is a negative interaction between NO and EDHF synthase, so that, in the presence of NO, EDHF synthesis is suppressed. On the other hand, the inhibitory effect of L-NAME on flow-dependent dilation could only be observed when the activity of the CYP epoxygenase was blocked. This interaction between the two enzyme systems, in response to flow/shear stress, seems to be gender dependent, since it has not been found in arterioles from male WT mice (32). The residual portion of the response in the presence of L-NAME and MCZ was abolished by Indo, confirming further the essential contribution of prostaglandins in the response (Figs. 2 and 4), similar to that observed in male mice (32).

The question remains as to whether and to what extent NO or CYP metabolites are responsible for the mediation of flow-induced dilation in arterioles of WT mice in control conditions. We assumed that if NO is the primary mediator, then blocking cGMP with ODQ will affect the NO-mediated portion of the response without MCZ having an additional effect, since the activity of the CYP epoxygenase was blocked. This interaction between the two enzyme systems, in response to flow/shear stress, seems to be gender dependent, since it has not been found in arterioles from male WT mice (32). The residual portion of the response in the presence of L-NAME and MCZ was abolished by Indo, confirming further the essential contribution of prostaglandins in the response (Figs. 2 and 4), similar to that observed in male mice (32).

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Recently, such an acute inhibition by NO of EDHF-induced coronary arteriolar dilation to bradykinin in vivo has been reported (25). The underlying mechanism responsible for this feedback inhibition of EDHF synthesis by NO released to flow or agonists may be that NO interacts with the prosthetic heme group of CYP (4). Because in the present study the prostaglandin-mediated component of the flow response was not affected by NO, it is unlikely that a decreased endothelial Ca\(^{2+}\) concentration by NO, reducing phospholipase A\(_2\) activity, would be responsible for the NO-mediated inhibition of EDHF synthesis (3, 4).

On the basis of previous studies (13, 17, 23), we propose that the difference in hormonal status is responsible for the differences observed between arterioles of male and female mice. Because synthesis of NO and EDHF, in response to a variety of stimuli, appears to be dependent on an increase in the intracellular concentration of Ca\(^{2+}\) and the formation of a Ca\(^{2+}\)-calmodulin complex (24) and because the threshold endothelial concentration of Ca\(^{2+}\) required for the activation of phospholipase is greater than that required for the activation of eNOS (4, 27), it is conceivable that the increases in endothelial Ca\(^{2+}\) concentration and calmodulin synthesis by estrogen (18) activate not only the L-arginine-NO pathway but also the phospholipase A\(_2\)-CYP pathway when stimulated by shear stress, leading to an EDHF-mediated dilation, a response that does, however, not occur in vessels of male mice. Also, a positive correlation between the level of estrogen and smooth muscle membrane hyperpolarization has been demonstrated (15). Because there are no methods available to test CYP epoxygenase activity of microvessels in response to shear stress, a stimulus that affects vascular responses by means of specific signal transduction pathways different from those activated by other stimuli (e.g., vasoactive agents), we could not explore the difference, if any, in the activity of this enzyme in response to shear stress between the...
arterioles of male and female mice. However, we found substantial basal release of EETs from the arterioles, which was PPOH sensitive (Fig. 8), providing the biochemical identification of these arachidonic acid metabolites in the microvessels studied.

Endothelial mediators contributing to flow-induced dilation in arterioles of female eNOS-KO mice. Flow-induced dilation in arterioles of female eNOS-KO mice is solely mediated by metabolites of CYP, namely EETs, and is not affected by an inhibitor of cyclooxygenase (Fig. 6). The response is completely abolished by IBTX (Fig. 7), suggesting that the CYP-mediated dilation to flow/shear stress is dependent on hyperpolarization of vascular smooth muscle, via activation of large-conductance KCa channels.

The absence of NO may potentiate EDHF production in arterioles at two different levels: acutely, as in WT mice given l-NAME or, chronically, as in eNOS-KO mice (2, 4). Conversely, the chronic inhibition of EDHF synthesis by NO may result from a decreased expression of the gene for EDHF synthase. In lipopolysaccharide- or interleukin-1β-treated vessels, it was reported that an increased production of inducible NO transcriptionally downregulates the putative EDHF-forming enzyme (19). By the same token, the enhanced EDHF synthesis, due to the genetic loss of eNOS, may also counteract the synthesis or activity of cyclooxygenase, leading to an exclusively EDHF-mediated response. Indeed, similar results, i.e., a solely MCZ/PPOH-sensitive response, instead of an NO/prostanoid-mediated response, to flow/shear stress, have been obtained in gracilis muscle arterioles of female rats treated chronically with l-NAME (35).

It was recently shown (33) that the resting membrane potential of smooth muscle from arteries with large-conductance KCa channels.

In conclusion, the present study demonstrates that, in female WT mice, flow-induced dilation in skeletal muscle arterioles is mediated by endothelial NO and prostaglandins. When NO is inhibited, EDHF becomes the mediator of the response. In female eNOS-KO mice, the preserved flow-induced dilation is mediated exclusively by EDHF. These findings reveal a novel compensatory mechanism in arterioles of female mice evoked by the absence of NO, by which EETs/EDHF contribute to the maintenance of shear stress-sensitive regulation of skeletal muscle arterioles and, consequently, peripheral resistance.

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