Gender-specific compensation for the lack of NO in the mediation of flow-induced arteriolar dilation

Yuming Wu, An Huang, Dong Sun, John R. Falck, Akos Koller, and Gabor Kaley

Department of Physiology, New York Medical College, Valhalla, New York 10595; and University of Texas Southwestern Medical Center, Dallas, Texas 75235

Received 7 December 2000; accepted in final form 10 January 2001

THE ENDOTHELIUM PLAYS AN IMPORTANT role in the regulation of vascular tone via release of dilator mediators, including nitric oxide (NO), prostaglandins, and endothelium-derived hyperpolarizing factor (EDHF), the latter generally being characterized as a metabolite of arachidonic acid via cytochrome P-450 (CYP) epoxygenase (6, 13, 18, 19). One of the important local factors governing arteriolar tone is wall shear stress, which is the primary stimulus for release of endothelial NO, as well as prostaglandins, in vivo (14, 15).

There is but scant evidence regarding the release of EDHF in response to increases in shear stress elicited by flow, especially because its synthesis is believed to be inhibited by the other two endothelial mediators released to flow (1, 24). Indeed, there is increasing evidence suggesting a feedback inhibition on EDHF production by NO and/or prostaglandins (2, 20, 24), as well as potential interactions among these three endothelial mediators (16). Upregulation of one system in response to a suppression or deficiency of the others has also been demonstrated (3, 4, 20). Our previous studies have shown that, in skeletal muscle arterioles, flow-induced dilation in male wild-type (WT) mice is mediated by endothelial NO and prostaglandins, whereas it is mediated exclusively by prostaglandins in arterioles of male endothelial NO synthase gene-deficient (eNOS-KO) mice (22). In the same vessels isolated from female WT mice, EDHF, together with prostaglandins, participates in the mediation of flow-induced dilation when NO is acutely inhibited; however, EDHF is solely responsible for the maintenance of this response in female eNOS-KO mice (9).

The question, therefore, arose as to whether the gender-specific compensation involving flow-induced dilation in response to NO deficiency is a universal phenomenon or simply a species-specific response. In addition, whether the effects of a short-term adaptation to the absence of NO synthesis are any different from those caused by a genetic lack of eNOS is of interest. Accordingly, we designed experiments to be conducted on arterioles of male and female rats that were chronically treated with Nω-nitro-L-arginine methyl ester (l-NAME), an inhibitor of the synthesis of NO.

METHODS

Animals. Twelve-week-old Wistar rats (Charles River Laboratories, Wilmington, MA) were divided into four groups: control untreated male, l-NAME-treated male, untreated female, and l-NAME-treated female rats. Rats received...
Flow-induced dilations were assessed by using miconazole. The specific inhibitor of CYP epoxygenase (23) was dissolved in DMSO at 10^{-2} M and further diluted with physiological salt solution (PSS). L-NAME and ChTX were dissolved in saline at 10^{-2} M and further diluted with physiological salt solution (PSS). The highest concentration of DMSO in the chamber was 0.1% (vol/vol), which had no effect on vessel tone.

Calculations and statistics. Passive diameter (PD) was used to assess the active tone (%PD) generated by arterioles in response to intravascular pressure and to normalize the changes in diameter in response to increases in flow in each vessel. Values are means ± SE; n, the number of rats. Statistical significance was calculated by repeated-measures of ANOVA followed by the Tukey-Kramer multiple-comparison test. Student’s t-test was also used, as appropriate. Significance level was taken at P < 0.05.
Increasing flow from 0 to 25 μl/min elicited significant increases in diameter of arterioles from all four groups of rats (Fig. 1). The magnitude of flow-induced dilation of arterioles was comparable in untreated and treated rats of the same gender but was significantly greater in female than in male rats (∼89.8 ± 1.3 vs. 73.9 ± 1.3% at 25 μl/min), confirming our previous findings (11).

Arterioles of male rats. The endothelial mediators responsible for the mediation of flow-induced dilation in arterioles of male rats are summarized in Fig. 2, showing that in untreated rats (A), Indo or L-NAME alone inhibited flow-induced dilation by ∼50%. Combined administration of both inhibitors abolished the responses. In rats treated chronically with L-NAME (Fig. 2B), Indo eliminated the dilator responses to flow, revealing a solely prostaglandin-mediated flow-induced dilation as a consequence of L-NAME treatment.

Arterioles of female rats. The endothelial mediators responsible for the mediation of flow-induced dilation in arterioles of female rats are summarized in Figs. 3 and 4. A prostaglandin-mediated portion of flow-induced dilation in arterioles of untreated female rats was demonstrated by the fact that Indo inhibited the responses by ∼50% whether Indo was administered first or last among the inhibitors used (Fig. 3). The roles of NO and metabolites of CYP in the mediation of flow-induced dilation in vessels of untreated female rats were also assessed by using L-NAME and MCZ. The Indo-resistant portion of the response was partially inhibited by L-NAME or MCZ after L-NAME (Fig. 3, top and middle). On the other hand, unlike L-NAME, MCZ alone did not affect the Indo-resistant portion of the response, which, however, was eliminated by additional administration of L-NAME (Fig. 3, bottom), suggesting an interaction between NO and metabolites of CYP.

Figure 4 shows that, in female rats treated chronically with L-NAME, flow-induced dilation was independent of prostaglandins, since Indo had no effect on the response. Dilation, however, was eliminated by MCZ or PPOH (Fig. 4A), revealing the involvement of the CYP pathway in the mediation of the responses, as a consequence of chronic L-NAME treatment. When these arterioles were treated with ChTX, flow-induced dilation was abolished (Fig. 4B).
DISCUSSION

The present study demonstrates a gender-specific adaptation to the lack of NO in the mediation of endothelium-dependent flow-induced dilations in rat gracilis muscle arterioles. It also shows that although NO and prostaglandins participate equally in the mediation of flow-dependent responses in control rats of both genders, after chronic treatment with L-NAME, prostaglandins account solely for this response in vessels of male rats and EDHF in vessels of female rats. Also, EDHF contributes partially to the mediation of flow-induced dilation when NO synthesis is acutely inhibited in female control rats. These findings are consistent with our previous studies in WT and eNOS-KO mice (9, 22). The congruence between the present and our previous studies reveals that a deficiency of eNOS-derived NO activates gender-specific signal transduction pathways.

It was reported previously that the cardiovascular system adapts to an acute inhibition of NO synthesis in a manner that is different from that observed with a chronic lack of NO (5). Also, our previous studies demonstrated that, in skeletal muscle arterioles of eNOS-KO mice, endothelial cells adapt to the chronic lack of NO and maintain a normal or close-to-normal response to shear stress by upregulation of the synthesis of other mediators, which, however, operate in a heterogeneous fashion dependent on gender (9, 22). Given that compensatory mechanisms may play an important role in the maintenance of cardiovascular function and that sex hormones may participate in the control of this compensation, it was of interest to define

Fig. 3. Normalized diameter of gracilis muscle arterioles of untreated female rats \((n = 5\) for each group), as a function of perfusate flow, in the control condition and after administration of L-NAME, Indo, or miconazole (MCZ, \(2 \times 10^{-6} \text{ M}\)) in different sequence, alone and in combination. *Significant difference between the 2 curves.

Fig. 4. Normalized diameter of gracilis muscle arterioles of female L-NAME-treated rats \((n = 6\) for each group), as a function of perfusate flow, in the control condition, in the presence of Indo, MCZ, or PPOH (A), and in the presence of charybotoxin (ChTX, \(2 \times 10^{-8} \text{ M}\); B). *Significant difference from control and from the presence of Indo.

Fig. 4. Normalized diameter of gracilis muscle arterioles of female L-NAME-treated rats \((n = 6\) for each group), as a function of perfusate flow, in the control condition, in the presence of Indo, MCZ, or PPOH (A), and in the presence of charybotoxin (ChTX, \(2 \times 10^{-8} \text{ M}\); B). *Significant difference from control and from the presence of Indo.
the nature of the mechanisms by which vessels are capable of responding normally to flow/shear stress in the absence of NO. To this end, flow-induced dilation and the nature of the endothelial factors mediating this response were investigated in gracilis muscle arterioles of rats of both genders treated chronically with L-NAME.

SBP was significantly enhanced, resulting in a reflex attenuation of heart rate in L-NAME-treated rats. Also, plasma concentrations of NO2/NO3 were significantly reduced after chronic L-NAME treatment (Table 1). The PD of arterioles of L-NAME-treated rats was significantly smaller than that of untreated littermates, a finding similar to that in eNOS-KO mice (9, 22), whereas the active diameters and the basal tone of vessels were comparable in the vessels of the two groups (Table 2). In addition, the results showing attenuated basal tone (Table 2) and enhanced flow-induced dilation (Fig. 1) in arterioles of female compared with male rats are consistent with our previous findings (10–12) showing that the greater basal and stimulated release of endothelial NO, triggered by the presence of estrogen, is responsible for these differences. Interestingly, in the present study, the reduced basal tone and greater flow-induced dilation in female than in male rats seem not to be purely NO dependent, since they are also present in L-NAME-treated littermates. On the other hand, the similar magnitude of flow-induced dilation in treated and untreated rats of the same gender (Fig. 1) further supports our hypothesis that arterioles of skeletal muscle are able to compensate for the absence of NO to maintain dilator responses to shear stress.

Adaptation of arterioles of male rats. Our present findings are similar to those we reported previously (14) showing that endothelium-derived NO and prostaglandins are coreleased in gracilis muscle arterioles of untreated male rats in response to increases in flow, that endothelium-derived NO and prostaglandins are responsible for the ensuing vasodilation, and that inhibition of NO or prostaglandin synthesis reduces the dilation by ~50%. Combination of both inhibitors eliminated the responses (Fig. 2A). In contrast, in arterioles of male rats treated chronically with L-NAME, Indo completely eliminated flow-induced dilation, indicating that the response is solely mediated by enhanced release of dilator prostaglandins (Fig. 2B), a finding that corresponds to that observed in the same vessels of male eNOS-KO mice (22). Recently, we demonstrated that this compensatory upregulation of prostaglandin synthesis most likely involves inducible cyclooxygenase (unpublished observations). In keeping with the present findings, an upregulation of cyclooxygenase activity, as a consequence of chronic NO deficiency, has also been demonstrated in the dog coronary (3, 21) and rat mesenteric circulations (8).

Adaptation of arterioles of female rats. As for the endothelial factors responsible for the mediation of flow-induced responses in arterioles of female mice, results shown in Fig. 3 indicate that in untreated rats, apart from the coparticipation of NO and prostaglandins, CYP metabolites contribute, in part, to the mediation of the responses, as indicated by the inhibitory effect of MCZ on the dilations. However, such a role for CYP metabolites was observed only in the presence of L-NAME, since MCZ alone did not affect the responses (Fig. 3, bottom). Unlike MCZ, either L-NAME or Indo alone significantly inhibited flow-induced dilation (Fig. 3, top and middle), indicating that NO and prostaglandins are the primary mediators of this response in normal conditions. The absence of NO after the acute administration of L-NAME activates CYP, eliciting EDHF formation, which then contributes to the mediation of flow-dependent dilation. A similar effect of a NO donor on agonist-induced EDHF release from porcine (1, 2) and canine coronary arteries (20) was also reported.

In female rats treated chronically with L-NAME (Fig. 4), MCZ or PPOH alone abolished flow-induced dilation, indicating a solely CYP metabolite-dependent response, unaffected by inhibition of cyclooxygenase (Fig. 4A). Furthermore, the dilation is completely inhibited by ChTX (Fig. 4B), suggesting further that the CYP-mediated dilation to flow is indeed dependent on hyperpolarization of vascular smooth muscle, via activation of Ca2+-sensitive K+ channels (6).

These results, together with those observed in eNOS-KO mice, may well form the basis of the gender-dependent mechanisms by which compensation for the lack of endothelial NO occurs in resistance vessels. Regarding the relationships between EDHF and female hormones, previous studies have provided some evidence suggesting that estrogen enhances the contribution of EDHF in the mediation of agonist-induced vasodilation (17) and smooth muscle membrane hyperpolarization (7). Further studies are necessary to establish the possible role(s) of specific hormones that are responsible for the activation of endothelial pathways leading to the synthesis of NO, prostaglandins, and EDHF in the two genders.

In conclusion, we demonstrated that, in skeletal muscle arterioles of male and female rats, NO and prostaglandins are the primary mediators of flow-induced dilation in control conditions. When NO synthesis is blocked acutely, EDHF participates in the responses of vessels of female rats. In arterioles of male rats chronically treated with L-NAME, prostaglandins are solely responsible for the preserved flow-induced dilation, whereas in vessels of L-NAME-treated female rats, this response is exclusively mediated by EDHF. These data, together with our previous findings in eNOS-KO mice, support our hypothesis that compensatory mechanisms in arterioles evoked by the absence of NO are indeed gender dependent in nature, through which enhanced contribution of endothelial mediators other than NO contribute to the maintenance of shear stress-sensitive regulation of skeletal muscle arterioles and, consequently, peripheral resistance.

We appreciate the excellent secretarial assistance of Miriam Nunez and Dana M. Spencer.
This study was supported by National Heart, Lung, and Blood Institute Grants HL-43023 and HL-46813, and American Heart Association Grant 9930244N.

REFERENCES


