Crucial Role of NO and Endothelium-Derived Hyperpolarizing Factor in Human Sustained Conduit Artery Flow-Mediated Dilatation

Jeremy Bellien, Michele Iacob, Laurence Gutierrez, Marc Isabelle, Agnes Lahary, Christian Thuillez, Robinson Joannides

Abstract—Whether NO is involved or not in sustained conduit artery flow-mediated dilatation in humans remains unclear. Moreover, the role of endothelium-derived hyperpolarizing factor (EDHF), synthesized by cytochrome epoxygenases and acting through calcium-activated potassium channels, and its relationship with NO during flow-mediated dilatation have never been investigated previously. In 12 healthy subjects we measured radial artery diameter (echotracking) and blood flow (Doppler) during flow-mediated dilatation induced by gradual distal hand skin heating (34 to 44°C), during the local infusion of saline and inhibitors of NO synthase (N^G-monomethyl-L-arginine [L-NMMA]: 8 to 20 μmol/min per liter), calcium-activated potassium channels (tetraethylammonium chloride: 9 μmol/min per liter), and cytochrome epoxygenases (fluconazole: 0.4 to 1.6 μmol/min per liter), alone and in combination. Mean wall shear stress, the flow-mediated dilatation stimulus, was calculated at each level of flow, and the diameter–wall shear stress relationship was constructed. During heating, compared with saline, the diameter–shear stress relationship was shifted downward by L-NMMA, tetraethylammonium, fluconazole, and, in a more pronounced manner, by the combinations of L-NMMA with tetraethylammonium or with fluconazole. Therefore, maximal radial artery flow-mediated dilatation, compared with saline (0.62±0.03 mm), was decreased under our experimental conditions by L-NMMA (−39±4%), tetraethylammonium chloride (−14±4%), fluconazole (−18±6%), and to a greater extent, by the combinations of L-NMMA with tetraethylammonium (−64±4%) or with fluconazole (−71±3%). This study demonstrates that NO and a cytochrome-related EDHF are involved in peripheral conduit artery flow-mediated dilatation in humans during sustained flow conditions. Moreover, the synergistic effects of the inhibitors strongly suggest a functional interaction between NO and EDHF pathways. (Hypertension. 2006;48:1088-1094.)

Key Words: conduit arteries ■ endothelium ■ flow-mediated dilatation ■ nitric oxide ■ endothelium-derived hyperpolarizing factor ■ cytochrome P450

Conduit artery flow-mediated dilatation (FMD) is a fundamental mechanism that regulates vascular conductance at rest and during exercise, as well as maintaining wall shear stress within physiological values. Vasodilatation is related to the integrity of the endothelium that releases vasodilatating factors in response to the increase in shear stress.1 In humans, we2 and others3,4 have demonstrated previously the major contribution of NO to peripheral conduit artery FMD in response to reactive hyperemia after a brief period of distal ischemia. This test is currently used in clinical experiments as an index of endothelial function and NO availability.5,6 However, recent data suggest that, during more sustained hyperemia, conduit artery FMD may occur independently of NO release.7–9 In fact, the local inhibition of NO synthesis by N^G-monomethyl-L-arginine (L-NMMA) in healthy subjects did not affect radial artery FMD induced by hand skin heating or after a prolonged period of ischemia.2 However, this result could be related to the duration of L-NMMA infusion that may have been insufficient to achieve an effective inhibition of NO synthase. Therefore, this does not definitively rule out a role for NO in sustained FMD. Moreover, in this context, if NO plays no role, the other endothelial factors involved in sustained conduit artery FMD should be identified. Furthermore, it was shown that protacyclin, derived from endothelial cyclooxygenase, plays no role in radial artery FMD during both sustained and nonsustained flow stimulations under physiological conditions nor during acute NO synthase inhibition.27 In contrast, whether or not an endothelium-derived hyperpolarizing factor (EDHF) is involved in sustained conduit artery FMD has never been investigated in humans.

Although different EDHFs exist depending on the species and vascular bed studied, their common mechanism of action...
is related to the opening of vascular calcium-activated potassium ($K_Ca$) channels promoting smooth muscle cell hyperpolarization and relaxation. In arterioles of NO-deficient mice, a recent study has demonstrated that FMD is mediated by epoxyeicosatrienoic acids (EETs), which are synthesized by endothelial cytochrome P450 (CYP) epoxygenases and diffused from the endothelium to activate muscular large $K_Ca$ channels. Similarly, in coronary arterioles obtained from patients undergoing cardiac surgery, an EDHF derived from CYP plays an important role in FMD and compensates for the loss of NO synthesis during coronary artery disease to maintain this response. In humans, in vivo, despite some controversial results, it has been shown that a CYP-dependent vasodilator mechanism and NO interact to regulate the exercise-induced increase in skeletal muscle blood flow suggesting the release of a CYP-related EDHF under these conditions. Regarding the conduit arteries, ex vivo experiments have shown that 11,12-EET can mediate the endothelium-dependent dilatation of human internal mammary arteries in response to acetylcholine. Moreover, we have reported recently that $K_Ca$ channels and CYP are involved in the regulation of basal radial artery diameter in healthy subjects suggesting a role for a CYP-related EDHF in human conduit arteries in vivo.

In this context, the aims of the present study were to evaluate, in vivo: (1) the role of NO in sustained peripheral conduit artery FMD by continuously infusing cumulative doses of the NO synthase inhibitor during the entire procedure; (2) the contribution of an EDHF derived from CYP epoxygenases by inhibiting its production and target channels during sustained conduit artery FMD; and (3) the relation of this EDHF with NO during this response, in the radial artery, using the hand skin heating method and the diameter–wall shear stress relationship.

Methods

Subjects

The study was performed in 12 male healthy volunteers on 3 separate days with a 2- to 3-week washout period between each experiment. Seven of these subjects also completed the protocol concerning the role of NO and EDHF under basal conditions reported previously. All of the subjects were normotensive, normolipemic, non-diabetic, nonsmoker, and deemed healthy on the basis of their medical history and complete medical examination with a normal ECG and routine laboratory tests (Table 1). None of the volunteers were taking medication at the time of the study. The forearm volume of each subject was measured by using the water displacement method based on the Archimedes’ principle, and drug infusion rates were normalized to 1-L tissue forearm by alteration of the drug concentration in the solvent while the pump speed of infusion was kept constant. The protocol was approved by the Haute-Normandie Consultative Committee for the Protection of Persons Engaged in Biomedical Research, and all of the participants gave written informed consent.

Instrumentation

Measurements were performed while subjects were in a supine position, in a quiet air-conditioned room, maintained at a constant temperature (22 to 24°C). A 27-gauge needle was inserted, under local anesthesia (1% lidocaine), into the brachial artery of the nondominant arm to permit saline infusion (0.9%) and pharmacological agents at a constant rate (1 mL/min). Systemic arterial pressure and heart rate were measured by means of a brachial cuff oscillometric device (Dinamap 8103, Critikon). Radial internal diameter, blood flow, and digital arterial pressure were continuously obtained using a high-precision echotracking device coupled to a Doppler system (NIUS 02, Asulab) and a finger photoplethysmograph (Finapres System, Ohmeda) as described previously. The hand skin temperature was modified by use of a water-filled thermocontrolled device (Polystat 1, Bioblock Scientific). Heating consisted of gradually increasing step by step the water temperature from 34°C to 37°C, 40°C, and 44°C. Each level of temperature was maintained for 8 minutes to reach a steady state (Supplementary Figure I at http://hyper.ahajournals.org) and to measure radial artery parameters during the last minute of each step. Total blood viscosity was measured in each subject on the inclusion visit using a cone-plate viscometer (Ex100 CTB, Brookfield) at a shear rate of 241 sec$^{-1}$ at 37°C. From the individual values of radial artery internal diameter (d), blood flow (Q), and viscosity ($\mu$), the mean arterial wall shear stress, the stimulus of FMD, was calculated at each level of temperature assuming a Poiseuillean model, that is, $\tau=(4\mu Q)/(r^3)$, ($r=d/2$) and the diameter–mean wall shear stress relationship was constructed. Reactive oxygen species (ROS) were quantified in local blood samples by electron paramagnetic resonance spectroscopy (Miniscope MS-200, Magnettech) in 6 additional healthy volunteers during a control hand skin heating performed without infusion (please see the expanded Methods section, available online).

Pharmacological Inhibitors Infused

During the 3 days of experiments, the subjects received saline used as control and 5 inhibitory treatments: L-NMMA (Clinalfa); an NO synthase inhibitor, tetraethylammonium chloride (TEA, Clinalfa), a nonspecific inhibitor of vascular $K_Ca$ channels; fluconazole (Pfizer Holding France), a potent inhibitor of the CYP epoxygenase 2C9; and the combination of L-NMMA with TEA; and the combination of L-NMMA with fluconazole. These treatments were administered in a randomized partial block design taking into consideration the long lasting effect of TEA (Table 1, available online). Each inhibitor was infused alone, and L-NMMA was also administered in combination with TEA and with fluconazole to evaluate a potential interaction between NO and EDHF pathways during radial artery FMD. The inhibitors were infused continuously during the entire heating procedure. Moreover, to obtain high cumulative doses and to compensate for the diluting effect of the increase in flow during heating (from 10 to 50 mL/min in control conditions), we increased the dose at the end of each temperature stage. Therefore, the starting dose of L-NMMA of 8 $\mu$mol/min per liter was infused during 8 minutes (64 $\mu$mol) at 34°C. This dose has been shown to fully abolish the maximal radial artery dilatation to high doses of acetylcholine without affecting systemic hemodynamics. Then, L-NMMA was infused at 12 $\mu$mol/min per liter at 37°C, 16 $\mu$mol/min per liter at 40°C, and 24 $\mu$mol/min per liter at 44°C.}

### Table 1. Demographic and Clinical Characteristics of the Healthy Volunteers Explored

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Totals (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
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<tr>
<td>Body mass index, kg/m$^2$</td>
<td>22.7±0.6</td>
</tr>
<tr>
<td>Systolic arterial pressure, mm Hg</td>
<td>120±3</td>
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<tr>
<td>Diastolic arterial pressure, mm Hg</td>
<td>66±2</td>
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<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>84±2</td>
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<tr>
<td>Heart rate, bpm</td>
<td>64±4</td>
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<tr>
<td>Glycemia, mmol/L</td>
<td>4.89±0.12</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>3.91±0.18</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.56±0.16</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.09±0.09</td>
</tr>
<tr>
<td>Triglyceridemia</td>
<td>0.58±0.05</td>
</tr>
<tr>
<td>Total blood viscosity, cP</td>
<td>4.78±0.09</td>
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</tbody>
</table>

Results are the mean±SEM of 12 subjects.
per liter at 40°C, and 20 μmol/min per liter at 44°C, 8 minutes each leading to a cumulative dose of 448 μmol, and a calculated local concentration ranging between 600 and 800 μmol/L. Similarly, flucnazole was infused at a dose of 0.4 μmol/min per liter at 34°C, 0.8 μmol/min per liter at 37°C, 1.2 μmol/min per liter at 40°C, and 1.6 μmol/min per liter at 44°C to obtain a local concentration ranging between 20 and 40 μmol/L, ~5 times higher than the in vitro inhibition constant of CYP 2C9, identified as EDHF synthase in humans,16,17 and with a weaker activity on other CYP enzymes.18,22 Finally, the dose of TEA was maintained at 9 μmol/min per liter to reach a local concentration ranging between 0.2 and 1 mmol/L that specifically inhibits single KCa channels in arterial smooth muscle cells without affecting the behavior of other potassium channels.18,20,21 In addition, this dose is 50% higher than the one significantly inhibiting the increase in forearm blood flow in response to bradykinin, but the maximal cumulative dose administered during 1 day (360 μmol) is lower than the intravenous dose affecting systemic hemodynamics (640 μmol).18,20,21

**General Procedure**

All of the subjects were evaluated in the Department of Pharmacology at 8:00 AM in the morning, 1 hour after a fat-free breakfast without tea or coffee. After instrumentation, saline was infused, and oral aspirin (500 mg) was administered to block vascular cyclooxygenase activity and exclude a role for prostacyclin in the responses observed.18,20,25 The hand was introduced into the thermostated device, and the temperature was fixed at 34°C to establish baseline conditions. Thirty minutes after aspirin administration, saline was continued (control conditions) or an inhibitory treatment was infused during 8 minutes. Then, sodium nitroprusside (SNP: 10 nmol/min per liter, 3 minutes) was immediately infused to assess the endothelium-independent dilatation of the radial artery.18 After a 20-minute rest at 34°C, radial artery FMD was evaluated in response to hand skin heating under baseline conditions (saline infusion) or during the concomitant and continuous infusion of the same inhibitory treatment than before SNP. After 1 hour resting and return to basal radial artery diameter and flow, the same procedure of SNP infusion and heating was repeated with another inhibitory treatment.

**Statistical Analysis**

Results are expressed as mean±SEM. Statistics were performed using the SYSTAT package (SYSTAT 5.2.1, SPSS). Analysis of the increase in flow with temperature was performed by repeated-measures ANOVA with inhibitors and subjects as factors and was followed, when significant, by a modified paired t test adjusted for multiple comparisons. The diameter–shear stress relationships obtained at different levels of flow were compared using an ANCOVA with subjects and inhibitors as factors and shear stress as a covariate and was followed, when significant, by a contrast analysis to compare the mean shift of the curves between inhibitors. The effects of the inhibitors on the maximal increase in radial artery diameter during heating (from 34 to 44°C) and on the radial artery endothelium-independent dilatation were assessed using ANOVA with subjects and basal radial artery diameter as factors. A value of P<0.05 was considered statistically significant.

**Results**

The systemic hemodynamical and radial artery parameters measured at baseline were homogeneous between the 3 days of exploration (Table 2). None of the inhibitors affected arterial pressure or heart rate (Table 3).

**Effects of the Inhibitors on Radial Artery Flow at Baseline and During Hand Skin Heating**

Before distal skin heating, at 34°C, the basal radial artery flow, compared with saline (8.6±0.8 mL/min), was similar during the infusion of flucnazole (9.0±0.8 mL/min; P value not significant) but was lower during the infusion of L-NMMA (6.9±1.2 mL/min; P<0.05), TEA (7.3±0.6 mL/min; P<0.05), and the combinations of L-NMMA with TEA (6.1±0.3 mL/min; P<0.05) or with flucnazole (7.1±0.6 mL/min; P<0.05). In addition, the basal radial artery flow during the infusion of the combination of L-NMMA with TEA was lower compared with TEA or L-NMMA alone (both P<0.05).

During heating, the radial artery flow increased with temperature in all of the cases (Figure 1; all P<0.001). The increase in flow at each level of temperature, compared with saline, was not affected during the infusion of TEA but was enhanced by flucnazole (P<0.05). In contrast, the increase in flow was similarly reduced during the infusion of L-NMMA alone or combined with TEA or with flucnazole (all P<0.05).

**Effects of the Inhibitors on Radial Artery Diameter at Baseline and During Hand Skin Heating**

Before distal skin heating, at 34°C, the basal radial artery diameter, compared with saline (2.70±0.07 mm), was similar during the infusion of L-NMMA (2.67±0.07 mm; P value not significant) or flucnazole (2.67±0.09 mL/min; P value not significant) but was lower during the infusion of TEA (2.59±0.06 mm; P<0.05) and the combinations of L-NMMA with TEA (2.51±0.05 mm; P<0.05) or with flucnazole (2.59±0.08 mm; P<0.05). In addition, the basal radial artery diameter during the infusion of the combination of L-NMMA with TEA was lower compared with TEA alone (P<0.05).

During heating, the radial artery diameter increased with mean wall shear stress in all of the cases (Figure 2; all P<0.001). Compared with saline, there was a downward shift of the diameter–mean wall shear stress relationship during the infusion of L-NMMA, TEA, flucnazole, and the combinations of L-NMMA with TEA or with flucnazole (all P<0.05). In addition, the downward shift of the diameter–shear stress relationship was higher during the combinations of L-NMMA with TEA or with flucnazole compared with L-NMMA, TEA, or flucnazole alone, respectively (all P<0.05). However, no significant difference was observed between either combination concerning the magnitude of this downward shift (P value not significant). Thus, the maximal radial artery FMD (from 34°C to 44°C) was reduced during the infusion of L-NMMA (0.37±0.04 mm), TEA (0.53±0.07 mm), flucnazole (0.51±0.04 mm), and, in a more significant manner,

**TABLE 2. Systemic Hemodynamical and Radial Artery Parameters Measured at Baseline During the 3 Days of Assessment**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic arterial pressure, mm Hg</td>
<td>123±4</td>
<td>118±4</td>
<td>120±5</td>
</tr>
<tr>
<td>Diastolic arterial pressure, mm Hg</td>
<td>64±1</td>
<td>62±2</td>
<td>62±2</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>84±2</td>
<td>81±3</td>
<td>83±3</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>72±5</td>
<td>69±3</td>
<td>71±4</td>
</tr>
<tr>
<td>Radial artery diameter, mm</td>
<td>2.64±0.08</td>
<td>2.62±0.07</td>
<td>2.60±0.04</td>
</tr>
<tr>
<td>Radial artery flow, mL/min</td>
<td>8.8±1.0</td>
<td>9.3±0.8</td>
<td>9.0±1.5</td>
</tr>
</tbody>
</table>

Results are the mean±SEM of 12 subjects.
during the infusion of the combinations of L-NMMA with TEA (0.22±0.04 mm) or with fluconazole (0.18±0.02 mm) as compared with saline (0.62±0.03 mm; Figure 3; all P<0.05).

Effects of the Inhibitors on the Endothelium-Independent Dilatation

SNP induced an increase in radial artery diameter in all of the cases (Figure 4; all P<0.001). The increase in radial artery diameter, compared with saline (0.50±0.05 mm from 2.64±0.07 mm), was similarly enhanced by L-NMMA (0.59±0.05 mm from 2.65±0.07 mm; P<0.05) and the combinations of L-NMMA with TEA (0.71±0.07 mm from 2.42±0.07 mm; P<0.05) and with fluconazole (0.63±0.059 mm from 2.48±0.05 mm; P<0.05) but was not significantly modified by TEA (0.54±0.09 mm from 2.53±0.09 mm; P value not significant) and fluconazole alone (0.47±0.09 mm from 2.60±0.10 mm; P value not significant).

Quantification of ROS

The local blood concentration of ROS increased during the hand skin heating procedure from 29.6±2.9 at 34°C to 38.3±4.1 μmol/L at 44°C (P<0.05).

Discussion

The major findings of this study are 2-fold. First, the decrease in radial artery FMD in response to hand skin heating during NO synthase inhibition demonstrates that, even during sustained flow conditions, NO is involved in peripheral conduit FMD in humans. Secondly, the magnitude of the decrease in radial artery FMD in response to hand skin heating during the inhibition of K<sub>Ca</sub> channels and CYP before and after NO synthase inhibition demonstrates that a CYP-related EDHF is involved in physiological conditions in sustained FMD and interacts functionally with the NO pathway to partially maintain this response.

The present study, performed on the radial artery, a model of peripheral conduit artery, was designed to demonstrate the role of NO in humans and to explore the physiological role of EDHF in conduit artery FMD during sustained flow conditions. We, therefore, used the method of hand skin heating to induce a sustained and gradual increase in radial artery blood flow that allows us to accurately estimate the radial artery FMD at each level of stimulus by the construction of the diameter–mean wall shear stress relationship. Furthermore, we evaluated the effects of the pharmacological inhibitors used in the present study on radial artery FMD taking into consideration their simultaneous effects on basal radial artery diameter and flow and subsequently on the flow stimulus of FMD, the variation in radial artery mean wall shear stress.

Regarding resistance arteries, at baseline before heating, the radial artery flow was less during L-NMMA and TEA administration confirming the role of NO and vascular K<sub>Ca</sub> channels in the control of the basal forearm vascular resistance in humans. In addition, as reported previously, the absence of decrease in flow after fluconazole shows that the basal activity of K<sub>Ca</sub> channels at this level is not modulated by a CYP-related EDHF. During local skin heating, there was an increase in regional blood flow that has been shown to be limited to the skin with no dilatation of the underlying muscular vascular bed and without an effect on the central thermoregulation. Under these conditions, the infusion of L-NMMA reduced the hyperemic response demonstrating the role of NO in the regulation of the skin arteriolar dilatation to local heating according to previous results performed using laser-Doppler flowmetry.
more, the blockade of $K_{Ca}$ channels by TEA did not modify the hyperemic response suggesting that an EDHF is not involved in skin arteriolar dilatation to heating. In contrast, fluconazole, in fact, markedly enhanced the skin arteriolar dilatation to heating demonstrating the involvement of an unexpected CYP-dependent vasoconstrictor mechanism in this response. This mechanism has never been reported in healthy subjects where CYP inhibition does not modify or reduce the dilatation of muscular resistance arteries in response to pharmacological stimuli or exercise, suggesting a heterogeneity between these territories and skin arterioles.13–16 In contrast, CYP inhibition has been shown to enhance forearm endothelium-dependent dilatation in patients with coronary artery disease through the reduction in the production of ROS by CYP epoxygenases leading to an increase in NO availability.15,28 In the present study, the increased hyperemia observed with fluconazole also depended on NO availability, because L-NMMA abolished the effect of CYP inhibition on the increase in flow.15 Furthermore, the increase in ROS production that we observed during heating strongly supports a role for ROS in the regulation of skin NO availability. Additional dedicated experiments are required to demonstrate that the skin production of ROS is CYP dependent.

Concerning the conduit artery, at baseline before heating, the radial artery diameter was similar after L-NMMA and fluconazole alone but was less after TEA alone and, to a greater extent, after both combinations. This result is consistent with our previously reported study that demonstrated an interaction between NO and a CYP-related EDHF to maintain this diameter.18 Furthermore, L-NMMA potenates radial artery dilatation to SNP demonstrating the hypersensitivity of the smooth muscle cells to exogenous NO and, therefore, the effective inhibition of NO synthesis in the arterial wall.18,24 Under these conditions, in the presence of L-NMMA, heating induced a lesser increase in radial artery diameter at each level of shear stress as compared with saline demonstrating that NO is involved in peripheral conduit artery FMD in humans during sustained flow conditions. This result contrasts with a previous study by Mullen et al7 who reported no significant effect of L-NMMA infusion on radial artery FMD during hand skin heating (from 34°C to 44°C) induced by L-NMMA, TEA, the combination of L-NMMA with TEA, fluconazole, and the combination of L-NMMA with fluconazole as compared with saline. $^{*}P<0.05$ vs baseline; $^{†}P<0.05$ vs each inhibitor alone.

![Figure 2. Radial artery diameter–mean wall shear stress relationships obtained during hand skin heating in the 12 healthy subjects under baseline conditions (saline) and during the concomitant infusion of L-NMMA (A), TEA alone and associated with L-NMMA (B), and fluconazole alone and associated with L-NMMA (C). $P<0.001$: time effect. $^{*}P<0.05$ vs saline; $^{†}P<0.05$ vs each inhibitor alone (inhibitor×shear stress interaction).](image)

![Figure 3. Bar graphs show the percentage decrease in radial artery FMD during hand skin heating (from 34°C to 44°C) induced by L-NMMA, TEA, the combination of L-NMMA with TEA, fluconazole, and the combination of L-NMMA with fluconazole as compared with saline. $^{*}P<0.05$ vs baseline; $^{†}P<0.05$ vs each inhibitor alone.](image)

![Figure 4. Bar graphs show the variation of radial artery endothelium-independent dilatation in response to SNP (10 nmol/min per liter, 3 minutes) in control conditions (saline) and after the infusion of L-NMMA, TEA, the combination of L-NMMA with TEA, fluconazole, and the combination of L-NMMA with fluconazole. $^{*}P<0.05$ vs saline.](image)
during hand warming. These conflicting results could be the consequence of the type of L-NMMA infusion protocol used. In fact, the cumulative dose of L-NMMA administered in the present study was >2 times higher, and L-NMMA was continuously infused throughout the entire procedure of heating and could, thus, have inhibited the NO syntheses recruited during the prolonged hyperemic stimulation. In addition, the population explored in the study reported by Mullen et al is certainly characterized by a low level of NO availability, because L-NMMA no longer reduced basal radial artery flow, whereas this type of constriction is usually observed in healthy humans.2–4,18,20,24,30 A similar phenomenon could explain the absence of effect of L-NMMA on the proximal coronary artery FMD in subjects with chest pain syndrome and normal angiograms.9,31

In this context, the infusion of TEA alone induced a downward shift of the diameter–shear stress relationship during heating. This effect of TEA was not related to a modification in the ability of the smooth cells to relax, because the blockade of vascular KCa channels did not modify the radial artery dilatation to SNP as reported previously both at the arteriolar level20 and in peripheral conduit arteries.18 Moreover, no residual effect of NO synthesis inhibition could be involved, because this decrease in FMD appeared similar with or without previous infusion of L-NMMA (Figure II). Finally, although some experiments have demonstrated that NO can exert its vasodilator effect through the activation of KCa channels,32 it seems unlikely that NO activates these channels to regulate FMD under our experimental conditions, because the association of L-NMMA with TEA was found to reduce radial artery FMD to a greater extent than L-NMMA alone. Thus, this result demonstrates that KCa channels are involved in the regulation of peripheral conduit artery FMD in vivo in humans during sustained flow stimulations in addition to NO. The contribution of KCa channels in the vasodilator response to the increase in shear stress is in accordance with previous ex vivo experiments performed in both arterioles11,12 and conduit arteries.8,33 Similarly, the administration of fluconazole shifted the diameter–shear stress relationship downward, demonstrating that a CYP-dependent vasodilator pathway is involved in sustained peripheral conduit artery FMD in humans. In fact, as stressed with TEA, this effect is not related to a modification in smooth muscle cell sensitivity18 or a long-lasting effect of previously infused L-NMMA (Figure II). Moreover, a direct interaction between fluconazole and KCa channels, described previously with some CYP inhibitors,34 seems unlikely in the present study, because the effects of fluconazole and TEA on basal radial artery parameters and hyperemia are in opposition.18 Because TEA and fluconazole reduced the radial artery FMD in a similar manner, we can reasonably conclude that an EDHF is synthesized by CYP and activates KCa channels to regulate peripheral conduit artery FMD in humans in vivo during sustained flow conditions. In this respect, ex vivo experiments have previously suggested the involvement of a CYP-related EDHF in the radial artery dilatation in response to pharmacological stimuli35 and in the endothelium-mediated dilatation in response to shear stress in arterioles of both animals11 and humans.12

Furthermore, the reduction of radial artery FMD during both combinations of infusion was 70% under our experimental conditions and, thus, seems to be more prominent than the addition of the effects of the inhibitors when infused alone, that is, 40% for L-NMMA plus 15% for TEA or fluconazole. These synergic effects strongly suggest a functional interaction between NO and EDHF pathways. Thus, during the loss of NO synthesis, a compensatory increase in the release of the CYP-related EDHF could occur in vivo in humans to partially maintain the sustained conduit artery FMD in accordance with numerous experimental data obtained in both resistance and conduit arteries.10–12,36–38

Finally, because only endothelium denudation or high concentration of KCl fully abolishes FMD, the persistent response observed in our study after the combined inhibitions could have resulted from the activation of distinct potassium channels or a fourth endothelial pathway.12,38 In addition, this remaining FMD could be related to the incomplete inhibition of NO synthesis and/or EDHF pathway. However, this seems unlikely with the local concentration of L-NMMA reached, because increasing the dose >100 μmol/L in animals does not reveal an NO-dependent component >50% when the combined inhibition with KCa channels provides >90% inhibition of sustained FMD.39 Conversely, our results may have underestimated the role of KCa channels.

Conclusions

This study demonstrates for the first time in humans that NO and a CYP-related EDHF play a crucial role in vivo in peripheral conduit artery FMD during sustained flow conditions and strongly suggests a functional interaction between both pathways to maintain this endothelium-dependent vasomotor response.

Perspectives

The exploration of the role of the CYP-derived metabolites and, in particular, of the EETs in pathology is of significant importance. In fact, EETs share many NO properties and, thus, represent an endogenous protective mechanism opposing the progression of cardiovascular diseases and, in particular, atherosclerosis.36,40 Additional experiments are warranted to fully characterize the balance between NO and EDHF, in humans, during sustained conduit artery FMD, to study the evolution of the balance in pathological states and to evaluate the impact of the alteration in EDHF availability on the prognosis of diseases. This could lead to the development of new pharmacological approaches to enhance vascular protection in cardiovascular diseases.

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