Endothelium-Derived Hyperpolarizing Factor
Identification and Mechanisms of Action in Human Subcutaneous Resistance Arteries

Paul Coats, PhD, BSc; Fiona Johnston, BSc; John MacDonald, MD, MRCP, BSc; John J.V. McMurray, MD, FRCP, FESC, BSc; Chris Hillier, PhD, BSc

Background—Both a vascular endothelial cytochrome P450 (CYP450) product of arachidonic acid metabolism and the potassium ion (K⁺) have been identified as endothelium-derived hyperpolarizing factors (EDHFs) in animal vascular tissues. We studied the relative importance of EDHF, nitric oxide (NO), and prostacyclin (PGI₂) as vasodilators in human subcutaneous arteries. We also examined the mechanisms underlying the vasodilator action of EDHF to elucidate its identity.

Methods and Results—Subcutaneous resistance arteries were obtained from 41 healthy volunteers. The contribution of EDHF to the vasodilation induced by acetylcholine was assessed by inhibiting production of NO, PGI₂, and membrane hyperpolarization. The mechanisms underlying the relaxation evoked by K⁺ and EDHF were elucidated. EDHF was found to account for ≈80% of acetylcholine-mediated vasorelaxation. Its action was insensitive to the combination of barium and ouabain, whereas barium and ouabain reversed K⁺-mediated vasorelaxation. EDHF-mediated vasorelaxation, however, was sensitive to the phospholipase A₂ inhibitor oleyloxyethyl phosphorylcholine and the CYP450 inhibitor ketoconazole.

Conclusions—EDHF is the major contributor to endothelium-dependent vasorelaxation in human subcutaneous resistance arteries. A product of phospholipase A₂/CYP450-dependent metabolism of arachidonic acid and not K⁺ is the likely identity of EDHF in human subcutaneous resistance arteries. (Circulation. 2001;103:1702-1708.)

Key Words: endothelium-derived factors ■ nitric oxide

Endothelial dysfunction is known to occur in a number of cardiovascular diseases, including atherosclerosis, hypertension, and heart failure. Therefore, considerable interest is attached to understanding the mechanisms underlying endothelium-dependent vasorelaxation. It is hoped that therapeutic strategies may be developed to counter vascular endothelial dysfunction in these and other diseases. The vascular endothelium modulates local blood flow via the dynamic release of numerous vasoactive factors. Receptor-dependent agonists, such as acetylcholine, bradykinin, and substance P, indirectly relax vascular smooth muscle by inducing the release of the known endothelium-derived relaxing factors (EDRFs) nitric oxide (NO), prostacyclin (PGI₂), and endothelium-derived hyperpolarizing factor (EDHF). At present, there is no clear consensus on the identity of EDHF or the exact mechanisms by which EDHF relaxes vascular smooth muscle. Some evidence, however, demonstrates that the action of EDHF involves calcium-sensitive K⁺ channels (KCa), which are sensitive to the combined effects of the toxins apamin and charybdotoxin. To date, numerous studies have aimed to identify the nature of EDHF and its mechanisms of action. Experimental evidence suggests that the most likely candidates are the P450 metabolite of arachidonic acid metabolism, 11,12-epoxyeicosatrienoic acid (11,12-EET), and the potassium ion (K⁺). K⁺ has long been known to possess vasodilator properties. Recently, Edwards and colleagues proposed that K⁺, as an EDHF, was extruded from the endothelium into the myoendothelial gap, leading to activation of vascular smooth muscle inwardly rectifying potassium channels and Na⁺,K⁺-ATPase pumps. This in turn resulted in efflux of K⁺ from the vascular smooth muscle, inducing hyperpolarization and vasorelaxation. Other recent and compelling evidence, however, suggests that the CYP450 enzyme product 11,12-EET, derived from the endothelium-dependent metabolism of arachidonic acid, is an EDHF in porcine coronary arteries and hamster gracilis muscle artery. The evidence that K⁺ or a P450 enzyme product is an EDHF, however, remains controversial. Therefore, real uncertainty exists regarding the identification of EDHF.

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TABLE 1. Basic Clinical Details of Volunteers Who Provided Biopsies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>65±1</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>93±2.5</td>
</tr>
<tr>
<td>Urea, mmol/L</td>
<td>5.6±0.2</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.18±0.2</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.31±0.2</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>151±24</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>81±7</td>
</tr>
</tbody>
</table>

BP indicates blood pressure.

There have been few studies of EDHF in human arteries. Nonetheless, it appears that an endothelium-dependent non-NOS, nonprostanoid mechanism of vasorelaxation predominates in these arteries.30–34 To date, there has been no specific study of EDHF in human subcutaneous resistance arteries. Therefore, in this study we aimed to identify the mechanisms of action and the likely identity of EDHF in human small subcutaneous resistance arteries.

Methods

Patients and Vessel Preparation

The hospital ethics review committee approved the study, and each subject gave informed written consent. Clinical characteristics of the volunteers are given in Table 1. Healthy volunteers with no history of vascular disease, diabetes, hypertension, or renal impairment attended the Clinical Investigations Research Unit at the Western Infirmary, Glasgow. Subcutaneous biopsies were isolated from 41 biopsies and incubated in PSS in the presence of either (1) norepinephrine (1 μmol/L), (2) norepinephrine+acetylcholine (10 μmol/L), (3) norepinephrine+acetylcholine+L-NOARG (100 μmol/L), or (4) norepinephrine+acetylcholine+oxadiazoloquinoxalin (ODQ, 10 μmol/L). All experiments were in the presence of isobutylmethylxanthine (10 μmol/L). At the end of the incubation period, the tissues were immersed in liquid nitrogen, homogenized in 5% ethanol, and centrifuged at 3000 rpm for 15 minutes. The supernatant was extracted and cGMP determined by radioimmunoassay.39 The amount of protein in the centrifuged pellet was determined by Bradford’s assay.40

Pharmacological Protocols

All studies were carried out in arteries preconstricted with norepinephrine to 80% of maximum response. In experiments in which K+-modified PSS was used, the concentration of norepinephrine was reduced to maintain a preconstriction diameter similar to previous studies.35 Briefly, PSS was gassed with 95% O2/5% CO2, with pH maintained at 7.4 at 37°C. Functional viability was assessed by maximum vasoconstriction to 60 mmol/L K’PSS and norepinephrine (10 μmol/L) and vasorelaxation (>80%) to acetylcholine. All arteries fulfilled these criteria, and none were discarded.

Relative Importance of EDHF

CCRCs were constructed with acetylcholine alone and after cumulative incubation with 100 μmol/L L-arginine (L-NOARG) for 1 hour, 30 μmol/L indomethacin for 30 minutes, and 25 mmol/L K’PSS.36–38

Identification of K’ Channels

All subsequent acetylcholine experiments were carried out with arteries preincubated with 100 μmol/L L-NOARG+30 μmol/L indomethacin. CCRCs were then constructed after intraluminal incubation with either (1) glibenclamide (10 μmol/L); (2) apamin (100 nmol/L); (3) charybdotoxin (100 nmol/L); (4) apamin+indomethacin. CCRCs were then constructed with acetylcholine alone and after cumulative concentration-response curves (CCRCs).

Role of Arachidonic Acid

Acetylcholine CCRCs were constructed after intraluminal incubation with isobutylmethylxanthine (OOPC) and ketoconazole. Endothelial specificity of these inhibitors was assessed by repeating CCRCs with sodium nitroprusside, pinacidil, and 1-ethyl-2-benzimidazoline (EBIO) in the presence of maximal concentrations of OOPC and ketoconazole.

Mechanisms of K’-Mediated Vasorelaxation

In standard PSS, the extracellular K’ concentration ([KCl]o) was elevated from 4.6 to 20 mmol/L in 2 mmol/L steps. This protocol was then repeated in the presence of BaCl2 or ouabain and Ba2+/ouabain.

Determination of cGMP Content

Human subcutaneous artery segments (~3 mm) were isolated from 3 subcutaneous biopsies and incubated in PSS in the presence of either (1) norepinephrine (1 μmol/L), (2) norepinephrine+acetylcholine (10 μmol/L), (3) norepinephrine+acetylcholine+L-NOARG (100 μmol/L), or (4) norepinephrine+acetylcholine+oxadiazoloquinoxalin (ODQ, 10 μmol/L). All experiments were in the presence of isobutylmethylxanthine (10 μmol/L). At the end of the incubation period, the tissues were immersed in liquid nitrogen, homogenized in 5% ethanol, and centrifuged at 3000 rpm for 15 minutes. The supernatant was extracted and cGMP determined by radioimmunoassay.39 The amount of protein in the centrifuged pellet was determined by Bradford’s assay.40

Drugs and Solutions

Acetylcholine, isobutylmethylxanthine, indomethacin, L-NOARG, norepinephrine, pinacidil, EBIO, and sodium nitroprusside were purchased from Sigma Chemical Co; apamin from Calbiochem; charybdotoxin from Bachem; ketoconazole from Tocris Cookson Ltd; and OOPC from Affinity Research. Anti-cGMP was a gift from Dr David Bunton (Glasgow Caledonian University, UK). PSS composition (in mmol) was NaCl 119, KCl 4.5, NaHCO3, 25, KH2PO4, 1.0, MgSO4·7H2O 1.0, glucose 11.0, and CaCl2 2.5. K’ PSS composition (25 and 60 mmol/L) was equimolar substitution of NaCl with KCl.

Data and Statistical Analysis

Relaxation data are represented as relaxation relative to the preconstricted diameter of the artery. Values are presented as mean±SEM. Statistical comparisons of pEC50 (concentration required to produce 50% of the maximum response) and maximum response were performed with Student’s paired t test followed by multiple comparisons by Bonferroni’s test where appropriate. Not calculable (NC) appears where the pEC50 could not be determined. Comparison of
CCRCs was by one-way ANOVA for repeated measures. Statistical significance was assumed at a value of $P<0.05$.

**Results**

The effects of L-NOARG, indomethacin, and 25 mmol/L K$^+$ PSS on acetylcholine-mediated vasorelaxation are shown in Figure 1. Incubation with L-NOARG significantly reduced both the maximum relaxation and $pEC_{50}$ (137±24 versus 72±14 nmol/L, $P<0.05$) to acetylcholine. Subsequent incubation with indomethacin failed to modify this response further. Exchange of PSS with 25 mmol/L K$^+$ PSS, however, abolished the relaxation response to acetylcholine (Figure 1).

Figure 2 summarizes the results of the cGMP radioimmunoassay. As expected, acetylcholine substantially increased cGMP generation. Incubation with L-NOARG or ODQ abolished the acetylcholine-dependent liberation of cGMP. Both L-NOARG (100 µmol/L) and ODQ (10 µmol/L) were equipotent at inhibiting the generation of cGMP.

Incubation with 10 µmol/L glibenclamide had no effect on the acetylcholine-dependent response (Figure 3). Apamin or charybdotoxin alone also had little effect on the response to acetylcholine (Figure 4). Incubation with the combination of apamin and charybdotoxin, however, abolished the relaxation to acetylcholine. Substitution of charybdotoxin with iberiotoxin had no effect on the relaxation response (Figure 5).

Cumulative addition of KCl on preconstricted arteries initially produced a concentration-dependent relaxation (4.6 to 14 mmol/L). This was reversed at higher concentrations (16 to 20 mmol/L) to a vasoconstriction response (Figure 6). Ba$^{2+}$ (30 µmol/L) or ouabain (1 mmol/L) alone was unable to significantly modify the K$^+$-mediated responses (data not presented). When combined, however, Ba$^{2+}$ and ouabain reversed K$^+$-induced vasorelaxation, resulting in vasoconstriction at all concentrations of KCl (Figure 6). In contrast, Ba$^{2+}$ and ouabain either alone (data not shown) or combined, at concentrations that reversed K$^+$-mediated vasorelaxation, had no effect on acetylcholine-mediated vasorelaxation (Figure 7).

The phospholipase A$(2)$ inhibitor OOPC reduced both sensitivity and maximum relaxation responses to the L-NOARG/
indomethacin-insensitive component of acetylcholine-mediated vasorelaxation in a concentration-dependent manner (Figure 8; EC50: ACh, 0.2 ± 0.1 μmol/L; +30 μmol/L OOPC, 1.0 ± 0.6 μmol/L; +100 μmol/L OOPC, NC; P < 0.05, 30 μmol/L OOPC versus ACh). OOPC had no effect on the sensitivity to norepinephrine, because the concentration used for preconstriction remained relatively constant. Furthermore, OOPC failed to inhibit the response to cumulative addition of sodium nitroprusside (Table 2). Incubation with the highest OOPC concentration (100 μmol/L) failed to modify the relaxation response to the ATP-sensitive K+ channel opener pinacidil and the Ca2+-sensitive K+ channel opener EBIO (Table 2).

The cytochrome P450 inhibitor ketoconazole also resulted in a concentration-dependent inhibition of the L-NOARG/indomethacin-insensitive relaxation to acetylcholine (Figure 9; EC50: ACh 0.3 ± 0.1 μmol/L; +1 μmol/L ketoconazole, 1.0 ± 0.6 μmol/L; +10 μmol/L ketoconazole, 3.5 ± 1.7 μmol/L; +30 μmol/L ketoconazole, NC; +100 μmol/L ketoconazole, NC; P < 0.05, 1 μmol/L ketoconazole versus ACh and 10 μmol/L ketoconazole versus 1 μmol/L ketoconazole). Again, ketoconazole did not modify the sensitivity of the tissue to the preconstricting agonist norepinephrine. Also, sodium nitroprusside–, EBIO–, and pinacidil-dependent responses were unaffected by the highest concentration of ketoconazole (100 μmol/L, Table 3).

Discussion

This study has 2 important findings. First, it demonstrates that EDHF in human subcutaneous resistance arteries is the major component of endothelium-dependent vasorelaxation. Second, a product of arachidonic acid metabolism, probably a cytochrome P450 product rather than the potassium ion, is likely to be an EDHF in these arteries.

A number of studies have suggested that a substance independent of NO and PGI2 is the major EDRF(s) in resistance arteries. Our results demonstrate that the L-NOARG/indomethacin-insensitive component of acetylcholine-mediated relaxation is the major component of endothelium-dependent relaxation in human subcutaneous resistance arteries. The NO component, ie, the L-NOARG–sensitive component, accounted for only ~20% of the maximum relaxation response to acetylcholine. Furthermore, indomethacin had no effect on the endothelium-dependent relaxation, indicating that PGI2 plays no role in the relaxation response in these ex vivo conditions. In contrast, the major component of the endothelium-dependent relaxation was sensitive to 25 mmol/L K+ PSS. This sensitivity to high

Subjects and Methods

TABLE 2. Sensitivity (pEC50) and Maximum Relaxation Response to Pinacidil (n = 3), Sodium Nitroprusside (SNP, n = 3), and EBIO (n = 3) Before and After Incubation With 100 μmol/L OOPC

<table>
<thead>
<tr>
<th>Additive</th>
<th>pEC50, nmol/L</th>
<th>Maximum Relaxation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinacidil</td>
<td>160 ± 120</td>
<td>78 ± 9</td>
</tr>
<tr>
<td>Pinacidil + 100 μmol/L OOPC</td>
<td>190 ± 140</td>
<td>78 ± 8</td>
</tr>
<tr>
<td>SNP</td>
<td>40 ± 30</td>
<td>93 ± 2</td>
</tr>
<tr>
<td>SNP + 100 μmol/L OOPC</td>
<td>51 ± 48</td>
<td>96 ± 3</td>
</tr>
<tr>
<td>EBIO</td>
<td>NC</td>
<td>86 ± 4</td>
</tr>
<tr>
<td>EBIO + 100 μmol/L OOPC</td>
<td>NC</td>
<td>84 ± 4</td>
</tr>
</tbody>
</table>
resistance arteries. This indicates that K<sub>ATP</sub> channels have no mediated relaxation to acetylcholine in human subcutaneous cerebral arteries, had no measurable effect on EDHF- shown to inhibit EDHF-mediated vasorelaxation in rabbit section. Our results confirm that the EDHF-mediated charybdotoxin together abolish the EDHF-mediated relaxation to acetylcholine; when combined, however, apamin and intermediate-conductance calcium-sensitive K<sub>Ca</sub> channels (BK<sub>Ca</sub>/IK<sub>Ca</sub>) alone have little effect on the EDHF-mediated relaxation in human subcutaneous resistance arteries. This indicates that K<sub>ATP</sub> channels have no role in this response.

One of the few points of consensus to emerge in EDHF research has been that preincubation of arteries with either apamin, an inhibitor of large-conductance calcium-sensitive K channels (BK<sub>Ca</sub>), or charybdotoxin, an inhibitor of intermediate-conductance calcium-sensitive K channels (IK<sub>Ca</sub>), alone has little effect on the EDHF-mediated relaxation to acetylcholine; when combined, however, apamin and charybdotoxin together abolish the EDHF-mediated relaxation. Our results confirm that the EDHF-mediated relaxation in human subcutaneous resistance arteries is similarly resistant to apamin or charybdotoxin alone but is abolished by the combination of the 2 toxins. Interestingly, substitution of charybdotoxin with iberiotoxin, a selective inhibitor of BK<sub>Ca</sub> in the presence of apamin had no effect on the relaxation response.

![Figure 9](https://example.com/figure9.png)

**Figure 9.** Vasorelaxation to acetylcholine (ACh) in presence of 100 μmol/L L-NOARG+30 μmol/L indomethacin after luminal incubation with P450 enzyme inhibitor ketoconazole (KETO). ANOVA for repeated measures, comparison of curves: *P<0.05, ACh response vs previous ACh response (n=6).

In vessels in which NO synthase and cyclooxygenase-1 are inhibited, glibenclamide, a K<sub>ATP</sub> channel inhibitor previously shown to inhibit EDHF-mediated vasorelaxation in rabbit cerebral arteries, had no measurable effect on EDHF-mediated relaxation to acetylcholine in human subcutaneous resistance arteries. This indicates that K<sub>ATP</sub> channels have no role in this response.

**TABLE 3. Sensitivity (pEC<sub>50</sub>) and Maximum Relaxation Response to Pinacidil (n=3), Sodium Nitroprusside (SNP, n=3), and EBIO (n=3) Before and After Incubation with 100 μmol/L Ketoconazole (KETO)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>pEC&lt;sub&gt;50&lt;/sub&gt;, mmol/L</th>
<th>Maximum Relaxation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinacidil</td>
<td>147±115</td>
<td>78±9</td>
</tr>
<tr>
<td>Pinacidil+100 μmol/L KETO</td>
<td>160±130</td>
<td>78±8</td>
</tr>
<tr>
<td>SNP</td>
<td>52±41</td>
<td>92±4</td>
</tr>
<tr>
<td>SNP+100 μmol/L KETO</td>
<td>38±50</td>
<td>93±3</td>
</tr>
<tr>
<td>EBIO</td>
<td>NC</td>
<td>87±4</td>
</tr>
<tr>
<td>EBIO+100 μmol/L KETO</td>
<td>NC</td>
<td>89±3</td>
</tr>
</tbody>
</table>

Difficult. Inhibition of IK<sub>Ca</sub> or BK<sub>Ca</sub>, alone clearly does not inhibit the actions of EDHF, suggesting that there is a BK<sub>Ca</sub>/IK<sub>Ca</sub> channel codependency involved in this mechanism of vasorelaxation. Alternatively, there may be an as yet unidentified BK<sub>Ca</sub>/IK<sub>Ca</sub>-like heteromultimeric K<sup>+</sup> channel associated with an EDHF-mediated response. Charybdotoxin has inhibitory action at BK<sub>Ca</sub> and a number of voltage-sensitive K<sup>+</sup> channels. The fact that charybdotoxin, as opposed to iberiotoxin, is required in combination with apamin to block the relaxation response insensitive to L-NOARG and indomethacin suggests that Ca<sup>2+</sup>-sensitive K<sup>+</sup> channels and voltage-sensitive K<sup>+</sup> channels are involved in this response.

Elevation of the extracellular potassium concentration, [K<sup>+</sup>]<sub>L</sub>, has been shown to dilate isolated arteries from rat cerebral, coronary, hepatic, and mesenteric vascular beds. The mechanism of this response involves K<sup>+</sup> efflux from vascular smooth muscle via the inwardly rectifying potassium channel (IK<sub>IR</sub>) and the Na<sup>+</sup>,K<sup>+</sup>-ATPase pump, leading to hyperpolarization and vasorelaxation. Using the protocols established by Edwards and colleagues, we mimicked the effects of EDHF by increasing [K<sup>+</sup>]<sub>L</sub>. In our study, elevation of [K<sup>+</sup>]<sub>L</sub> resulted in a small vasorelaxation of preconstricted arteries. The maximum relaxation response occurred at ≈14 to 16 mmol/L [K<sup>+</sup>]<sub>L</sub>; increasing [K<sup>+</sup>]<sub>L</sub> further resulted in vasoconstriction. The [K<sup>+</sup>]<sub>L</sub>-dependent vasodilation observed on the combination of Ba<sup>2+</sup>, an inhibitor of K<sub>IR</sub> plus ouabain, an inhibitor of the Na<sup>+</sup>,K<sup>+</sup>-ATPase pump, but not Ba<sup>2+</sup> or ouabain alone. In fact, elevation of [K<sup>+</sup>]<sub>L</sub>, in the presence of Ba<sup>2+</sup> plus ouabain resulted in a concentration-dependent vasoconstriction at all increments of [K<sup>+</sup>]<sub>L</sub> and in all arteries studied. This observation confirms K<sup>+</sup> as having vasodilator properties and confirms the role of the K<sub>IR</sub> and the Na<sup>+</sup>,K<sup>+</sup>-ATPase pump in K<sup>+</sup>-dependent vasodilation in these vessels. Ba<sup>2+</sup> plus ouabain, however, had no effect on the EDHF-mediated relaxation to acetylcholine. This observation shows that EDHF is insensitive to inhibition of the K<sub>IR</sub> and the Na<sup>+</sup>,K<sup>+</sup>-ATPase pump.

These results demonstrate that the EDHF component of the acetylcholine-mediated endothelium-dependent relaxation is mediated via mechanisms different from those through which K<sup>+</sup> mediates vasorelaxation. If K<sup>+</sup> were an EDHF in human subcutaneous resistance arteries, this response should have been sensitive to the combination of Ba<sup>2+</sup> plus ouabain.

Phospholipase A<sub>2</sub>-dependent metabolism of membrane-bound phospholipids is a primary source of arachidonic acid and contributes to EDHF-mediated vasorelaxation in rabbit mesenteric arteries. In turn, the metabolism of arachidonic acid by P450 enzymes into vasoactive substances is a commonly identified phenomenon in vascular physiology. In the present study, luminal incubation with OO OPC, a specific inhibitor of phospholipase A<sub>2</sub>, had a profound effect on endothelium-dependent relaxation to EDHF. Likewise, luminal incubation with the P450 enzyme inhibitor ketoconazole resulted in a concentration-dependent inhibition. The OO OPC and ketoconazole results provide strong evidence that the endothelium-dependent relaxation to EDHF is a product
of phospholipase A₂/arachidonic acid/P450 enzyme metabolism in human subcutaneous resistance arteries.

We are confident that these results reflect specifically endothelium-dependent mechanisms, because the vasorelaxation response to the endothelium-independent vasodilator sodium nitroprusside was unaffected by the highest concentrations of OOPC and ketoconazole. Moreover, the endothelium-independent mechanisms of hyperpolarization were unaffected by this treatment, because the K⁺ᵢₛₚ channel opener pinacidil and the Ca²⁺-sensitive K⁺ channel opener EBI0 produced potent vasorelaxation unaffected by either OOPC or ketoconazole.

In this study, we were unable to measure smooth muscle membrane potential and therefore have no direct evidence that the acetylcholine-mediated relaxation insensitive to L-NOARG and indomethacin is definitely the result of hyperpolarization. Raising [K⁺]ₜ₉ to 25 mmol/L, however, completely blocked the L-NOARG/indomethacin-insensitive response to acetylcholine. Previous studies have shown that raising [K⁺]ₜ₉ has little effect on NO and PGI₂-mediated vasorelaxation, but rather it specifically antagonizes the actions of EDHF by counterbalancing smooth muscle cell membrane potential.

Experimental evidence suggests that the commonly used concentrations of NO synthase inhibitors may not be completely effective in blocking all NO production. This is important, because NO has been shown to have a hypopolarizing effect via cGMP in vascular smooth muscle and the response insensitive to L-NOARG may be as a consequence of residual NO production. L-NOARG (100 μmol/L) in this tissue, however, abolished the acetylcholine-dependent liberation of cGMP. Therefore, we are confident that there was no residual NO and that the relaxation responses observed were independent of NO. Moreover, NO has been shown to hyperpolarize vascular tissue via cGMP- and cAMP-dependent mechanisms sensitive to iberiotoxin and glibenclamide, respectively. Both iberiotoxin and glibenclamide had no effect on the relaxation response insensitive to L-NOARG and indomethacin. Therefore, we are confident that the acetylcholine-mediated relaxation insensitive to L-NOARG and indomethacin is truly an NO/prostanoid-dependent phenomenon and is likely to be mediated by an EDHF.

This study shows that endothelium-dependent relaxation to EDHF is mediated via mechanisms different from those mediating K⁺ vasorelaxation; therefore, it is unlikely that K⁺ is an EDHF in human subcutaneous resistance arteries. Moreover, this mechanism is sensitive to phospholipase A₂ and P450 enzyme inhibition, thus indicating that EDHF in human subcutaneous resistance arteries is likely to be a P450 enzyme–dependent product of arachidonic acid metabolism. Also, vasodilation depends largely on K⁺ channels, suggesting that selective use of appropriate potassium channel drugs may provide a useful therapeutic approach for the treatment of vascular pathologies.

Acknowledgments

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References


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