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Cyclooxygenase Inhibition Restores Nitric Oxide Activity in Essential Hypertension

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Abstract To evaluate whether cyclooxygenase constrctor substances can impair nitric oxide–mediated vasodilation in essential hypertension, in seven normotensive subjects (43 ± 4 years, BP, 117 ± 6/81 ± 2 mm Hg) and seven essential hypertensive patients (47 ± 5 years, BP, 151 ± 8/98 ± 4 mm Hg), we studied forearm blood flow (strain-gauge plethysmography) modifications induced by intrabrachial acetylcholine (0.15, 0.45, 1.5, 4.5, 15 μg 100 mL−1 min−1) in basal conditions, during infusion of N°-monomethylnitric oxide (L-NMMA, 100 μg 100 mL−1 min−1), a nitric oxide synthase inhibitor, or indomethacin (50 μg 100 mL−1 min−1), a cyclooxygenase inhibitor, or simultaneous indomethacin and L-NMMA. In normotensives, vasodilation to acetylcholine was increased by L-arginine (maximum flow increase 539 ± 48%, and 386 ± 42%, respectively, P < 0.01), and this effect was unchanged by indomethacin. In contrast, in hypertensive patients, vasodilation to acetylcholine (maximum flow increase 671 ± 64%, and 386 ± 42%, respectively) was significantly decreased by L-arginine (maximum flow increase 635 ± 53% and 458 ± 33%) was unchanged by L-NMMA. Indomethacin significantly (P < 0.01) increased the response to acetylcholine (maximum flow increase 635 ± 53%) and restored the inhibitory effect of L-NMMA (maximum flow increase 445 ± 36%, P < 0.01 versus indomethacin alone). In an adjunctive seven normotensive patients but not in normotensive control subjects, impaired endothelium-dependent responses in cardiocascular disease such as essential hypertension. Thus, in essential hypertensive patients, vasodilation to endothelium-dependent agonists (mainly acetylcholine or bradykinin) is reduced compared with normotensive control subjects.13 This abnormality is caused by a defect in NO activity since both L-arginine and L-NMMA, which in normal conditions can increase or decrease the response to acetylcholine, respectively, are ineffective in hypertensive patients.16–18 Moreover, indomethacin, a cyclooxygenase inhibitor, can potentiate the vasodilation to acetylcholine in hypertensive patients, but not in normotensive control subjects, indicating the synthesis of cyclooxygenase-dependent EDCF.13 Thus taken together these observations suggest that in essential hypertension endothelial dysfunction is caused by the simultaneous presence of a defect in the L-arginine–NO pathway and production of cyclooxygenase-dependent EDCF.

Methods

Patients

The study population included 14 normotensive control subjects and 14 matched essential hypertensive patients. Subjects with hypercholesterolemia (total cholesterol greater than 5.2 mmol/L), diabetes mellitus, cardiac and/or cerebral ischemic vascular disease, impaired renal function and other major pathologies were excluded from the study. Moreover, subjects or patients smoking more than five cigarettes per day and/or consuming more than 60 g of ethanol (corresponding to half a liter of wine) per day were excluded from the study. In accordance with institutional guidelines, all patients were aware of the investigational

Key Words: hypertension • endothelium • nitric oxide • endothelium-derived factors • indomethacin
nature of the study and gave written consent to it. Any pharmacological treatment was discontinued for at least 2 weeks before performing the study.

Subjects, defined as normal according to the absence of familial history of essential hypertension and BP below 140/90 mm Hg, were characterized by mean age of 47.6±4.3 years and BP values of 115.6±6.1/80.3±2.1 mm Hg. Essential hypertensive patients were recruited from among the newly diagnosed cases in our outpatient clinics if they reported the presence of positive family history of essential hypertension, whenever supraventricular BP after 10 minutes of rest) measured by mercury sphygmomanometer three times at 1-week intervals was consistently found greater than 140/90 mm Hg. Secondary forms of hypertension were excluded by routine diagnostic procedures. Mean age was 50.6±6.6 years and BP values were 152.5±8.7/99.2±3.6 mm Hg. Since the patients were newly diagnosed cases, they were never treated and the known history of hypertension had lasted 2±0.4 years. The demographic and clinical characteristics of the two groups are shown in the Table.

### Experimental Procedure

All studies were performed at 0800 AM after overnight fast with the subjects lying supine in a quiet air-conditioned room (22°C to 24°C). A polyethylene cannula (21 gauge, Abbott) was inserted into the brachial artery under local anesthesia (2% lidocaine) and connected through stopcocks to a pressure transducer (Model MS20, Electromedics) for systemic mean BP (one third pulse pressure + diastolic pressure) and heart rate monitoring (Model VSM1, Physiocontrol) and for intra-arterial infusions. FBF was measured in both forearms (experimental and contralateral forearm) by strain-gauge venous plethysmography (LOOSCO, GL LOOS).

Circulation to the hand was excluded by a pediatric cuff around the wrist at suprasystolic BP. Details concerning the sensitivity and reproducibility of the method as performed in our laboratory have already been published.

Forearm volume was measured according to the water displacement method and drug infusion rates were normalized to 100 mL tissue by alteration of the drug concentration in the solvent while the pump speed of infusion was kept constant. Drugs were infused at systematically ineffective rates through separate ports via three-way stopcocks.

### Characteristics of Study Subjects (mean±SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive Subjects (n=14)</th>
<th>Essential Hypertensive Patients (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>47.6±4.3</td>
<td>50.6±6.6</td>
</tr>
<tr>
<td>Age range, y</td>
<td>39-56</td>
<td>37-81</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>9/5</td>
<td>10/4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>72±4.1</td>
<td>71±5.2</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>115±6.1</td>
<td>152±5.8</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>80±3.2</td>
<td>99±3.5</td>
</tr>
<tr>
<td>Cardiac mass, g/m²</td>
<td>110±7.1</td>
<td>116±4.8</td>
</tr>
<tr>
<td>Plasma glucose, mg/dL</td>
<td>82±5.6</td>
<td>89±5.9</td>
</tr>
<tr>
<td>Plasma total cholesterol, mg/dL</td>
<td>189±12.3</td>
<td>193±12.8</td>
</tr>
<tr>
<td>Plasma HDL cholesterol, mg/dL</td>
<td>42±6.8</td>
<td>40±6.4</td>
</tr>
<tr>
<td>Plasma LDL cholesterol, mg/dL</td>
<td>118±10.2</td>
<td>121±11.3</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>21.8±0.5</td>
<td>22.1±0.6</td>
</tr>
<tr>
<td>FBF, mL 100 mL⁻¹  min⁻¹</td>
<td>3.4±0.4</td>
<td>3.4±0.5</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein, LDL, low-density lipoprotein.

### Experimental Design

Endothelium-dependent vasodilation was estimated by performing a dose-response curve to intra-arterial acetylcholine (cumulative increase of the infusion rates 0.15, 0.45, 1.5, 4.5, 15 μg/100 mL forearm tissue per minute, for 5 minutes at each dose) while endothelium-independent vasodilation was assessed with a dose-response curve to intra-arterial sodium nitroprusside, a direct smooth muscle cell relaxant compound (cumulative increase by 1, 2, and 4 μg/100 mL forearm tissue per minute, for 5 minutes at each dose) These rates were selected to induce vasodilation comparable to that obtained with acetylcholine.

### Effect of Cyclooxygenase Inhibition on Response to Acetylcholine in the Presence of L-NMMA

In seven normotensive subjects and seven essential hypertensive patients, after sodium nitroprusside infusion, the dose-response curve to intra-arterial acetylcholine was performed according to the following design during saline (0.2 mL/min), in the presence of intra-arterial L-NMMA (100 μg/100 mL forearm tissue per minute, started 10 minutes before acetylcholine and continued throughout), in the presence of intra-arterial indomethacin (50 μg/100 mL forearm tissue per minute, started 10 minutes before acetylcholine and continued throughout), and finally in the presence of simultaneous infusion of L-NMMA and indomethacin.

### Effect of Cyclooxygenase Inhibition on Response to Acetylcholine in the Presence of L-Arginine

In an adjunctive seven normotensive subjects and seven essential hypertensive patients, the same previously described protocol was performed by replacing L-NMMA with L-arginine infused intrabrachially at 200 μg/100 mL forearm tissue per minute. Thirty minutes of washout was allowed between each dose-response curve in the presence of saline, while 60 minutes was allowed when L-arginine or indomethacin was infused simultaneously.

### Data Analysis

Since arterial pressure did not significantly change during the study, all data were analyzed in terms of FBF, FBF increments were taken as evidence of local vasodilation. Differences between two means were compared by paired or unpaired Student’s t test, as appropriate. Responses to acetylcholine and sodium nitroprusside were analyzed by ANOVA for repeated measures and Scheffe’s test was applied for multiple comparison testing. Results were expressed as mean±SD.

### Drugs

Acetylcholine HCl (Farmigea SPA), indomethacin (Lioneta- cei, Guest Farmaceutici SPA), L-arginine (Clinalfa AG),...
L-NMMA (Chinalfa AG), and sodium nitroprusside (Malesc) were obtained from commercially available sources and diluted freshly to the desired concentration by adding normal saline. Sodium nitroprusside was dissolved in glucosate solution and protected from light by aluminum foil.

**Results**

**Response to Intrabrachial Acetylcholine and Sodium Nitroprusside**

In the overall population, vasodilation to acetylcholine was significantly (P < 0.01) blunted in essential hypertensive patients (FBF rose from 3.4 ± 0.5 to a maximum of 17.1 ± 3.2 mL/100 mL forearm tissue per minute with the highest dose) compared with normotensive control subjects (FBF rose from 3.4 ± 0.4 to a maximum of 23.9 ± 5.4 mL/100 mL forearm tissue per minute with the highest dose) (Fig 1). In contrast, the vasodilating effect of the endothelium independent vasodilator sodium nitroprusside was similar in normotensive subjects and essential hypertensive patients (FBF rose from 3.6 ± 0.4 to a maximum of 24.2 ± 2.9 mL/100 mL forearm tissue per minute with the highest dose and from 3.5 ± 0.4 to a maximum of 23.3 ± 3.1 mL/100 mL forearm tissue per minute, respectively, NS) (Fig 1).

**Effect of Cyclooxygenase Inhibition on Response to Acetylcholine in the Presence of L-NMMA**

In this group of normotensive control subjects, L-NMMA infusion caused a decrement in basal FBF (from 3.4 ± 0.4 to 1.9 ± 0.2 mL/100 mL forearm tissue per minute; P < 0.01) and significantly blunted the vasodilating effect of acetylcholine (saline: from 3.4 ± 0.4 to 26.2 ± 5.6 mL/100 mL forearm tissue per minute, L-NMMA from 1.9 ± 0.2 to 8.3 ± 2.1 mL/100 mL forearm tissue per minute, P < 0.01 versus acetylcholine alone) (Fig 2). Again indomethacin did not change either basal FBF (from 3.4 ± 0.4 to 3.4 ± 0.4 mL/100 mL forearm tissue per minute), the response to acetylcholine (from 3.4 ± 0.4 to 26.8 ± 5.4 mL/100 mL forearm tissue per minute), or the inhibiting effect of L-NMMA on vasodilation to acetylcholine (from 1.9 ± 0.2 to 8.3 ± 2.5 mL/100 mL forearm tissue per minute) (Fig 2).

In the essential hypertensive patients, L-NMMA infusion caused a decrement in basal FBF (from 3.2 ± 0.5 to 2.3 ± 0.5 mL/100 mL forearm tissue per minute, P < 0.01) which was significantly smaller than that observed in normotensive control subjects (percent FBF decrease 44% versus 28%, respectively, P < 0.01). However, the response to acetylcholine (from 3.2 ± 0.5 to 17.7 ± 4.2 mL/100 mL forearm tissue per minute) was not changed by L-NMMA (from 2.3 ± 0.5 to 12.5 ± 3.2 mL/100 mL forearm tissue per minute, NS versus saline) (Fig 2). Indomethacin infusion did not change basal FBF (from 3.1 ± 0.4 to 3.1 ± 0.6 mL/100 mL forearm tissue per minute) Nevertheless, the cyclooxygenase inhibitor increased the response to acetylcholine (from 3.1 ± 0.4 to 22.0 ± 3.3 mL/100 mL forearm tissue per minute, P < 0.01 versus acetylcholine during saline) (Fig 2). Finally, when the effect of L-NMMA was tested in the presence of indomethacin, the NO synthase inhibitor blunted the vasodilating response to acetylcholine (from 2.3 ± 0.5 to 12.5 ± 3.7 mL/100 mL forearm tissue per minute, P < 0.01 versus acetylcholine during indomethacin alone) (Fig 2).
Different results were obtained in essential hypertensive patients. Again, acetylcholine infusion caused a dose-dependent vasodilation (from 3.6±0.5 to 16.4±2.9 mL/100 mL forearm tissue per minute) which was statistically lower (P<0.01) than that observed in normotensive control subjects. L-Arginine administration changed neither basal FBF (from 3.6±0.5 to 3.7±0.4 mL/100 mL forearm tissue per minute) nor the vasodilating effect of acetylcholine (from 3.7±0.4 to 16.5±3.1 mL/L/100 mL forearm tissue per minute, NS versus saline) (Fig 3). Indomethacin did not change basal FBF (from 3.3±0.5 to 3.4±0.4 mL/100 mL forearm tissue per minute), but significantly increased the response to acetylcholine (from 3.4±0.4 to 23.6±3.4 mL/100 mL forearm tissue per minute, P<0.01 versus saline) (Fig 2). It is worth noting, finally, that when L-arginine was coinfused with indomethacin the vasodilating effect of acetylcholine was further increased (from 3.4±0.4 to 31.1±4.1 mL/100 mL forearm tissue per minute, P<0.01 versus acetylcholine in the presence of indomethacin) (Fig 3).

In both normotensive subjects and essential hypertensive patients contralateral FBF did not significantly change during the whole study (data not shown).

**Discussion**

Essential hypertension is characterized by endothelial dysfunction.1-18 This alteration is further confirmed in the present study since the response to acetylcholine, an endothelium-dependent vasodilator,1,5,22 but not to sodium nitroprusside, a direct smooth muscle cell relaxant,23 was blunted in essential hypertensive patients compared with matched normotensive control subjects. The mechanisms responsible for the impaired endothelium-dependent vasodilation include an alteration in the L-arginine–NO pathway and production of cyclooxygenase-dependent EDCF. That these mechanisms can operate in essential hypertensive patients is confirmed by the present results. Thus, in agreement with previous observations,16 administration of L-arginine, the substrate for NO synthase,3 can increase the vasodilating effect of acetylcholine in normotensive subjects while the amino acid is ineffective in essential hypertensive patients. Moreover, L-NMMA, an antagonist for NO synthase,16 can blunt the response to acetylcholine in control subjects, but not in hypertensive patients.15 Taken together these results clearly confirm the presence of a defect in the endothelium-derived NO system in essential hypertension, since neither activation nor inhibition of the NO pathway can lead to modifications of the vascular response to the endothelium-dependent vasodilator. It is important to observe that the lack of effect of these compounds on acetylcholine-induced vasodilation is not linked to insufficient infusion rates of either L-arginine or L-NMMA, as already demonstrated by previous evidence obtained in similar experimental conditions.14,17

Moreover, this abnormality does not totally account for the impaired vasodilation to acetylcholine observed in essential hypertension. Thus in hypertensive patients, but not in normotensive subjects, indomethacin increased the response to the endothelium-dependent vasodilator, confirming that, in agreement with previous evidence,16 the production of cyclooxygenase derivatives can curtail endothelial responses in essential hypertension. It is worth noting that for the first time the alteration in the endothelium-derived NO system and production of cyclooxygenase-dependent EDCF has been demonstrated in the same
patients, supporting the possibility that these endothelial alterations coexist in essential hypertension.

However, the main finding of the present study is the demonstration that in essential hypertensive patients cyclooxygenase activity is not involved in the pathogenesis of hypertension. Indomethacin administration restores the potentiating and inhibiting effect of L-arginine and L-NMMA, respectively, on acetylcholine-induced vasodilation. In contrast, in normotensive control subjects, indomethacin does not change the effect of L-arginine and L-NMMA on endothelium-dependent vasodilation to acetylcholine. Taken together these findings suggest that in essential hypertension, cyclooxygenase activity causes endothelial dysfunction by producing cyclooxygenase-dependent substances which, at least partially, can inactivate the L-arginine–NO system. There is experimental evidence indicating that in vessels from hypertensive animals a close relationship exists between the L-arginine–NO pathway and cyclooxygenase activity. Thus in primary hypertension, cyclooxygenase activity is increased to produce not only vasoconstrictor prostanoids, but also superoxide anions, which cause NO breakdown.

Moreover, it has been demonstrated in canine basilar arteries that an increased production of superoxide anions can destroy NO produced by the activity of the L-arginine pathway, thus causing full expression of vasoconstrictor prostanoids. This negative interaction between the NO system and cyclooxygenase activity could be a likely explanation for the impaired endothelium-dependent vasodilation, which is characteristic of essential hypertensive patients. Therefore, in our experimental conditions, in essential hypertensive patients the stimulation of endothelial cells could cause the activation of two parallel pathways involving both NO synthase and cyclooxygenase. Cyclooxygenase activity could lead to the production of NO-inactivating substances (endoperoxides) superoxide anions?), thus explaining the absence of effects of L-arginine and L-NMMA on vasodilation to acetylcholine. When cyclooxygenase is blocked by indomethacin and NO breakdown no longer occurs or at least decreased, it is therefore possible to demonstrate the activity of L-arginine and L-NMMA as observed in normotensive control subjects.

The relationship between cyclooxygenase-dependent EDCF and primary hypertension is of interest. It must be noted that these substances, while causing endothelial dysfunctions, do not seem to contribute to an increase in BP values. Thus, in the spontaneously hypertensive rat, treatment by ifetroban, a thromboxane A2/prostaglandin endoperoxide-receptor blocker, normalized endothelium-dependent relaxations to acetylcholine in isolated segments of aorta but produced no reduction in BP values. In agreement with experimental data, in human hypertension the acute administration of ritodrine, a combined thromboxane synthase inhibitor and thromboxane A2 receptor antagonist, did not lower BP values in essential hypertensive patients. In addition, the lack of relationship between cyclooxygenase-dependent EDCF and BP values in humans is further confirmed by recent evidence indicating that production of such substances seems to be mainly related to the aging process. Thus in normotensive subjects it is possible to detect production of EDCF when aging increases over 60 years, and this phenomenon is antagonized in essential hypertensive patients (starting from the fourth decade of life). However, it is worth noting that in young essential hypertensive patients production of cyclooxygenase-dependent EDCF does not seem to occur.

Therefore, the dissociation between EDCF production and elevated BP values underlines the possibility that EDCF do not participate in the development of hypertension. However, it is well documented that whatever is the nature of EDCF (endoperoxide or superoxide anions), they can increase vascular tone and stimulate platelet aggregation or smooth muscle cell proliferation by direct mechanisms or by inducing NO breakdown. It is therefore conceivable that EDCF production, although not important as a causal mechanism responsible for the development of hypertension, probably plays a role in the vascular damage associated with aging and hypertension itself.

As regards the important issue of the relationship between the duration of essential hypertension and EDCF production, no data are available to understand whether the degree of synthesis of these substances is in some way dependent on the length of the hypertensive process. In the present study, unfortunately the recruited hypertensive population shows a quite short duration of hypertension and no conclusion can be drawn.

Finally, it is worth noting that these endothelial mechanisms operate mainly when endothelial cells are stimulated by acetylcholine. Thus, in agreement with previous observations, neither L-arginine nor indomethacin (nor the combination of both compounds) can influence basal blood flow in either normotensive subjects or essential hypertensive patients. In contrast, L-NMMA infusion can decrease basal FBF, confirming that NO is basally released in human vasculature and this mechanism is defective in essential hypertension since, as previously demonstrated, L-NMMA-induced vasodilation is blunted in hypertensive patients compared with control subjects. However, the simultaneous infusion of indomethacin with L-NMMA does not change the vasoconstrictor effect of the NO synthase inhibitor suggesting that cyclooxygenase activity does not participate in NO-mediated local regulation of basal flow.

In conclusion, the present results indicate that endothelial dysfunction which is characteristic of essential hypertension is determined by the simultaneous presence of an alteration in the L-arginine–NO pathway and production of cyclooxygenase derivatives. These alterations do not seem to be independent since in essential hypertensive patients, but not in normotensive control subjects, the dysfunction NO system seems to be restored or at least improved by cyclooxygenase blockade. Which cyclooxygenase-dependent substances could be responsible for inhibition of the L-arginine NO pathway is, at the present time, under investigation.

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