Nitric Oxide and Endothelin-1 are Important Regulators of Human Ophthalmic Artery

Ivan O. Haefliger,*† Josef Flammer,* and Thomas F. Lüscher,††

The vascular endothelial cells have the ability to modulate local vascular tone by releasing relaxing factors such as nitric oxide or the vasoconstrictor peptide endothelin-1. Although this regulatory system is found in all vertebrates, there is a great heterogeneity in the release of these endothelium-derived substances, from one organ to another, between large and small vessels, and between different species. Therefore, observations made in certain vascular beds or animals do not necessarily apply to human ophthalmic circulation. The present study was designed to investigate endothelial mediators in the human ophthalmic artery.

The results show that in the human ophthalmic artery, nitric oxide is released under basal conditions and that its production can be markedly stimulated by bradykinin, acetylcholine, and particularly histamine, which cause profound vascular relaxation. In contrast, endothelin-1 evoked potent contractions, which were unaffected by the calcium antagonist nifedipine. However, upon re-exposure of the blood vessels to the peptide, marked tachyphylaxis occurred.

These findings demonstrate that in the human ophthalmic artery, endothelium-derived nitric oxide and endothelin are very potent modulators of vascular tone, suggesting that they play an important role in the regulation of local blood flow in the eye. Hence, endothelium dysfunction may represent a new pathogenetic mechanism in disease states associated with altered blood flow to the eye, such as diabetes, hypertension, and some forms of low-tension glaucoma.

Materials and Methods. Human eyes with surrounding orbital tissues were obtained at the autopsy room 5–10 hr after death and put into cold modified Krebs-Ringer's solution (millimolar): NaCl, 118; KCl, 4.7; CaCl2, 2.5; MgSO4, 1.2; KH2PO4, 1.2; NaHCO3, 25; EDTA, 0.026; glucose, 11.1. Under a microscope, the ophthalmic artery was dissected free and cut into small rings (2 mm). Two tungsten wires (30 μm and 80 μm) were passed through their lumen and connected to a myograph system filled with control solution (37°C; 95% O2; 5% CO2). After 45 min of equilibration, the vessels were stretched to their optimal tension (550 ± 50 mg; n = 4). The function of the endothelium was considered normal if bradykinin (10−6 mol/l) or acetylcholine (10−6 mol/l) evoked more than 75% relaxation of a contraction to serotonin (3 × 10−7 mol/l).3,4 Out of 15 specimens obtained from the autopsy room, nine fulfilled these criteria. The mean age of these subjects was 64 ± 6 yr (range, 41–78), and the postmortem time averaged 6.5 ± 1.0 hr (range 5–10).

After a washout period, the relaxing effect of bradykinin (10−9–10−5 mol/l), acetylcholine (10−9–10−4 mol/l), and histamine (10−9–10−4 mol/l) was tested in preparations with endothelium by adding cumulative concentrations of the drugs on top of a half maximal contraction to serotonin (3 × 10−7 mol/l). Relaxations were expressed as percent of this response. To assess the contribution of nitric oxide, experiments were repeated (in parallel or in series) with the inhibitor of nitric oxide formation, nitro-L-arginine methyl ester (L-NNA 10−4 mol/l). In vessels without functional endothelium, cumulative concentration-response curves to endothelin-1 (10−11–10−7 mol/l) were constructed in parallel, in the absence or presence of nifedipine (10−7–10−5 mol/l). Contractions were expressed as percent of the maximal response to KCl (100 mmol/l). All experiments were conducted in the presence of indomethacin (10−5 mol/l) to inhibit the production of prostaglandins.3 All drugs were purchased from Sigma (St Louis, MO), except serotonin and endothelin-1, which were from Serva (Heidelberg, Germany) and Novabiochem (Läufelfingen, Switzerland), respectively. All drugs were dissolved in distilled water, except nifedipine (dissolved in ethanol). Concentrations are expressed as negative
Fig. 1. Nitric oxide formation in the human ophthalmic artery in response to bradykinin (left panel) and acetylcholine (right panel). The relaxations were markedly reduced or prevented in the presence of nitro-L-arginine methyl ester (L-NNA; ■), demonstrating that bradykinin and acetylcholine stimulate the release of nitric oxide.

molar concentration in the organ chamber solution. For statistical analysis, the area under the concentration response curves (arbitrary units: 0–1000) were calculated. Results are given as mean ± standard error of the mean. In all experiments, n equals the number of subject studied. Paired Student's t-tests or analysis of variance (ANOVA) followed by Scheffe's F-test were used for statistical comparison. A P value smaller than 0.05 was considered significant.

Results. In quiescent rings with endothelium, the inhibitor of nitric oxide formation, L-NNA 10^{-4} mol/l, induced a contraction of 127 ± 25 mg, (P < 0.01 vs control, n = 8) corresponding to 10% of the maximal response to KCl (100 mmol/l).

In rings precontracted with serotonin, bradykinin (10^{-9}–10^{-5} mol/l), acetylcholine (10^{-8}–10^{-4} mol/l; Fig. 1) and histamine (10^{-8}–10^{-5} mol/l; Fig. 2) evoked concentration-dependent relaxations. The maximal response averaged 85, 90, and 267%, respectively. The relaxations were markedly inhibited by L-NNA 10^{-4} mol/l (Figs. 1 and 2; P < 0.05 vs. control). The relaxations to histamine were markedly reduced by L-NNA and contractions were unmasked in the presence of L-NNA plus the H_{2}-histaminergic antagonist cimetidine (Fig. 2).

In quiescent rings, endothelin-1 (10^{-11}–10^{-7} mol/l) evoked concentration-dependent contractions comparable to those induced by a maximal concentration of KCl (100 mmol/l; Fig. 3 left). The maximal contraction to endothelin-1 was not sustained despite the continued presence of the peptide. After 2 hr of a slow decrease in tension, a plateau contraction was reached (Table 1). After a washout period, re-exposure to endothelin-1 induced only a very small contraction (Fig. 3 left; P < 0.001 vs. control). The contractile properties of the vessels were not affected because the response to KCl was unchanged (n = 6, not significant; data not shown).

The calcium (Ca^{2+}) antagonist nifedipine (10^{-7}–10^{-5} mol/l) did not affect the response to endothelin-1 (Fig. 3 right, Table 1), although the response to KCl...
Tachyphylaxis to the Peptide

Effect of the Calcium Antagonist Nifedipine

Fig. 3. Contractions to endothelin-1 in the human ophthalmic artery. Endothelin-1 caused marked contractions (○), which were markedly blunted after re-exposure to the peptide (●); left panel. The Ca²⁺ antagonist nifedipine (right panel, solid symbols) had no significant effects on the contractions evoked by the peptide.

(100 mmol/l) was reduced by the antagonist (Table 1; P < 0.05-0.01). Nifedipine did not affect the decrease in tension nor the plateau contraction that occurred after the addition of the maximal concentration of endothelin-1 (Table 1; not significant).

Discussion. The present study demonstrates the presence and profound influence of the endothelium-derived relaxing factor, nitric oxide, and the vasoconstrictor peptide endothelin in the human ophthalmic artery. Interestingly, the human ophthalmic artery exhibited a basal release of nitric oxide as the inhibitor of nitric oxide formation, L-NNA, caused significant contractions. This indicates that the human ophthalmic circulation normally is in a state of constant vasodilation resulting from the production of nitric oxide.

Furthermore, bradykinin, acetylcholine, and histamine markedly stimulated the release of nitric oxide. Indeed, L-NNA profoundly reduced the relaxation to the agonists. The response to acetylcholine and bradykinin were comparable to the responses occurring in the porcine ophthalmic artery. Although others have reported relaxations to acetylcholine in the human ophthalmic vascular bed, the present study demonstrates that the response is mediated by nitric oxide. In some blood vessels, inhibitors of nitric oxide do not markedly inhibit endothelium-dependent relaxations, most likely because of the concomitant release of an endothelium-derived hyperpolarizing factor. This obviously does not occur in human ophthalmic arteries. Particularly striking was the potency of histo-

Table 1. Calcium antagonists and endothelin-1 in the human ophthalmic artery*

<table>
<thead>
<tr>
<th>Endothelin-1</th>
<th>Area under the response curve (arbitrary units)</th>
<th>Maximal contraction (% of KCI)</th>
<th>Plateau contraction (% of KCI)</th>
<th>KCl (100 mM) (% of the first response)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>296 ± 60</td>
<td>82 ± 15</td>
<td>24 ± 7f</td>
<td>96 ± 9</td>
</tr>
<tr>
<td>Nifedipine 10⁻⁷ M (n = 6)</td>
<td>246 ± 27</td>
<td>71 ± 4</td>
<td>20 ± 5§</td>
<td>65 ± 7</td>
</tr>
<tr>
<td>Nifedipine 10⁻⁶ M (n = 6)</td>
<td>223 ± 42</td>
<td>63 ± 7</td>
<td>13 ± 4†</td>
<td>46 ± 9 §</td>
</tr>
<tr>
<td>Nifedipine 10⁻⁵ M (n = 6)</td>
<td>249 ± 32</td>
<td>65 ± 8</td>
<td>16 ± 4#</td>
<td>46 ± 9 §</td>
</tr>
</tbody>
</table>

* The dihydropyridine Ca²⁺ antagonist nifedipine did not affect contractions to endothelin-1, but markedly reduced those to potassium chloride (KCI). Contractions are expressed as percent of the maximal response to KCl (100 mM).
mine. Indeed, the blood vessels relaxed below baseline (indicating they do exhibit basal tone). The receptor involved in nitric oxide release evoked by histamine must be of the H<sub>2</sub> histaminergic subtype, while the H<sub>3</sub> histaminergic receptor (blocked by cimetidine) mediated a direct, nitric oxide-independent relaxation (as previously described in the human internal mammary artery).<sup>6</sup>

In the human ophthalmic artery, endothelin-1 evoked strong contractions, but the response was not mediated by influx of extracellular Ca<sup>2+</sup> through voltage-operated Ca<sup>2+</sup> channels, as the Ca<sup>2+</sup> antagonist nifedipine had no significant effect.<sup>5</sup> This contrasts with observations made in the bovine ophthalmic vascular bed, where the Ca<sup>2+</sup> antagonist nitrendipine abolished the contractions of retinal arteries induced by the peptide.<sup>7</sup> Hence, species differences also exist in the response to the endothelium-derived vasoconstrictor endothelin-1.

In contrast to other vascular beds,<sup>3</sup> the contraction induced by endothelin-1 was not sustained, and re-exposure to the peptide (after washout) only induced a very small contraction, even though the response to KCl (which mediates contractions independent of receptor-operated mechanisms) remained unaffected. This tachyphylaxis is explained best by a down-regulation of endothelin receptors and could represent an efficient protective mechanism of this vascular bed against repeated and prolonged exposure to endothelin-1.

In conclusion, in human ophthalmic arteries, the endothelial L-arginine/nitric oxide pathway is active under basal conditions and is further stimulated by bradykinin, acetylcholine, and histamine. The potency of these responses and the responses of endothelin-1 suggest an important physiological role for endothelium-derived vasoactive substances in the regulation of the human ophthalmic circulation. Furthermore, a dysfunction of these endothelial mechanisms, which occurs in diabetes<sup>3,8</sup> and hypertension (at least in peripheral arteries)<sup>3</sup> may play an important role in the pathophysiology of ophthalmic complications. In addition, in certain glaucoma patients with ocular vasospasms,<sup>8,10</sup> endothelial dysfunction may represent the underlying cause or at least contribute to alterations in ophthalmic blood flow.

**Key words:** bradykinin, acetylcholine, histamine, nitro-L-arginine methyl ester, nifedipine.

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**References**