Altered Endothelin-1 Vasoreactivity in Patients with Untreated Normal-Pressure Glaucoma

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Purpose. Vasospasm, resulting from a generalized dysfunction in the vascular endothelium, is implicated in the development of normal-pressure glaucoma (NPG). Impaired endothelium-derived nitric oxide activity and abnormalities of the endothelin system suggest systemic endothelial cell dysfunction in patients with NPG. Endothelin (ET)-1 vasoreactivity was assessed in the peripheral circulation of patients with NPG.

Methods. Forearm blood flow was measured using venous occlusion plethysmography in eight patients with untreated NPG and eight age- and sex-matched healthy volunteers during intra-arterial infusion of ET-1 (5 pmol/min) and, on a separate occasion, to BQ123, a selective endothelin-A receptor antagonist, (100 nmol/min). Blood pressure and heart rate were measured in the noninfused arm, and plasma ET-1 concentrations were measured using a radioimmunoassay.

Results. Forearm blood flow fell during infusion of ET-1 ($P < 0.001$ for both) to a similar extent in both groups ($P = 0.7$; patients versus control subjects). In contrast, BQ123 increased forearm blood flow in both groups ($P < 0.001$ for both), although the vasodilatation was lower in patients than in control subjects ($P < 0.001$; patients versus control subjects). There was no difference in basal plasma ET-1 concentrations between the two groups ($P = 0.81$; patients versus control subjects).

Conclusions. Despite normal responses to ET-1, patients with NPG have reduced vasodilatation in response to ET_A-receptor antagonism. This could be due to attenuated ET_A-receptor–mediated tone, increased ET_B-receptor–mediated contraction or impaired ET_B-receptor-mediated release of endothelial nitric oxide. These results are consistent with the authors' previous demonstration of systemic vascular dysfunction in patients with NPG. (*Invest Ophthalmol Vis Sci.* 2006;47:2528–2532) DOI:10.1167/iovs.05-0240

The increased prevalence of vasospastic disorders, such as Raynaud's phenomenon and migraine[1,2] in patients with normal-pressure glaucoma (NPG), suggests that this condition is an ocular manifestation of a generalized vascular defect.[3] This is supported by evidence of functional abnormalities in both the ocular (reduced blood flow velocity; increased resistance)[4,5] and systemic (reduced digital blood flow)[6,7] circulations of patients with NPG.

Vasospasm, characterized by exaggerated vascular responses to various stimuli such as temperature and stress,[8,9] is a transient, reversible vasoconstriction that results from impaired endothelium-dependent regulation of vascular tone.[10] Endothelial cell dysfunction produces an imbalance between vasodilator and vasoconstrictor pathways; most notably the nitric oxide (NO) and endothelin systems.[11] Endothelin (ET)-1 is a potent vasoconstrictor produced predominantly by endothelial cells.[12] It induces vasoconstriction by interaction with ET_A (and to a lesser extent ET_B) receptors on vascular smooth muscle cells but can also stimulate NO-mediated vasodilatation by activation of ET_B receptors on the endothelium.[13] Consequently, ET-1–induced vasoconstriction is subject to physiological antagonism by both basal and ET_B-mediated release of NO from the endothelium.[14] Interaction of ET-1 with both receptor subtypes contributes to maintenance of basal vascular tone.[15,16]

ET-1 has been implicated as a contributory factor in many vasospastic disease processes, including vasospastic coronary artery disease,[17] cerebral vasospasm and subarachnoid hemorrhage,[18,19] and Raynaud's disease.[20] The possibility that ET-1 contributes to vasospasm in NPG is supported by the demonstration of elevated basal plasma ET-1 concentrations in patients[21,22] combined with an abnormal response of plasma ET-1 concentrations to postural[23] and temperature changes.[24]

We have shown, using direct measurement of vascular function, that peripheral endothelium-dependent relaxation is impaired,[25] or altered,[26] in patients with NPG. Furthermore, enhanced ET-1-dependent contraction in isolated subcutaneous resistance arteries from these individuals suggests impaired ET_B-mediated release of endothelial NO.[27] ET-1–mediated contraction in vivo has not been assessed in NPG, however. This study investigated the hypothesis that ET-1–mediated endothelium-dependent relaxation is impaired in patients with NPG. The specific purposes of the present study were to determine whether, in patients with NPG, there is evidence of impaired vasoreactivity in response to ET-1.

Patients and Methods

Eight patients with newly diagnosed, untreated NPG and eight healthy volunteers, matched for age, sex, mean arterial blood pressure, weight, and forearm length, were recruited. NPG was diagnosed on the basis of the accepted criteria: mean intraocular pressure <22 mm Hg on diurnal phasing, gonioscopically open anterior chamber angle, characteristic optic disc cupping (cup-disc ratio >0.6) with either thinning or notching of the neuroretinal rim, and glaucomatous visual field loss detected by automated perimetry (Humphrey Instruments; Carl Zeiss Meditec, Dublin, CA) with the 24-2 threshold program (average mean deviation = −5.15 dB and corrected pattern standard deviation = 9.21 dB). Where indicated, neuroimaging was used to exclude an intracranial cause of disc or field changes. None of the patients had other ocular disease, or had previously received steroid therapy. All control subjects had normal findings in a normal ocular examination, normal

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Table 1. Baseline Characteristics of Patients with NPG and Matched Control Subjects

	NPG	Control	*P*
Sex (M:F)	5:3	5:3	
Age (y)	58.12 ± 10.74	59.37 ± 8.21	0.57
IOP (mm Hg)	17.81 ± 2.33	16.53 ± 3.32	0.51
MAP (mm Hg)	101.74 ± 15.46	98.32 ± 11.04	0.42
HR (beats/min)	64.62 ± 6.67	64.00 ± 5.26	0.31
Forearm length (cm)	24.2 ± 1.13	25.1 ± 2.1	0.62
Weight (kg)	73.1 ± 7.2	69.04 ± 6.72	0.94
CVS Disease	3	1	0.31
Vasospastic Disease	3	1	0.31
ET-1 (pg/mL)	2.39 ± 0.41	3.16 ± 0.54	0.81

Results are expressed as the mean ± SEM and were compared using unpaired *t*-test. M:F, male-to-female ratio; MAP, mean arterial blood pressure; HR, heart rate; CVS Disease, cardiovascular disease; ET-1, endothelin-1.

intraocular pressure, and normal visual fields. None of the subjects was taking any vasoactive medication. Approval was granted by the Lothian Research Ethics Committee and the tenets of the Declaration of Helsinki were upheld. Written, informed consent was obtained from all subjects before enrollment in the study.

Measurement of Forearm Blood Flow

Forearm blood flow was measured using the technique of venous occlusion plethysmography. This technique has been well described before.[25,28] Briefly, it allows measurement of responses of forearm resistance vessels to the local, intra-arterial infusion of vasoactive substances. These substances are infused into the brachial artery of the nondominant arm, with the contralateral arm acting as a contemporaneous control. Forearm blood flow is simultaneously recorded in both arms by measuring changes in forearm circumference using mercury-in-silastic strain gauges (D. E. Hokanson, Bellevue, WA) placed around the widest part of the forearm, after vascular occlusion achieved with two rapid cuff inflators (model E20; D. E. Hokanson). Data were processed and recorded with an appropriately calibrated computerized data-acquisition system (MacLab analog-to-digital converter with Chart version 3.3.9; AD Instruments, Castle Hill, Australia). Percentage change in the infused forearm blood flow is calculated using the following equation: percentage change in forearm blood flow = 100 × (It/Nit − Ib/Nib)/(Ib/Nib), where Ib and Nib are the infused and noninfused forearm blood flows at baseline (time 0) and It and Nit the infused and noninfused forearm blood flows at a given time.

Blood pressure and heart rate were recorded in the noninfused arm immediately after each blood flow measurement using a semiautomated, noninvasive oscillometric sphygmomanometer (model UA 751; Takeda Medical, Tokyo, Japan).

Measurement of Plasma ET-1 Concentrations

Venous blood was drawn from the antecubital vein. ET-1 was extracted from the plasma using the method of Rolinski et al.[29] and analyzed by radioimmunoassay (RIA) based on a commercially available kit (Peninsula Laboratories Inc., Bachem Group, San Carlos, CA). Briefly, the sample (100 μL) was incubated overnight with the appropriate antibody and then incubated with a known concentration of radiolabeled ET-1 for a further 16 hours. Immune complexes were then precipitated with donkey anti-rabbit antibody (Amerlex; GE Healthcare, Chalfont St. Giles, UK), counted in a gamma counter and concentrations interpolated from a standard curve. In our hands, this RIA has intra- and interassay variations of 6.3% and 7.2%, respectively. The reference range was 1.5 to 4.5 pg/mL, the sensitivity (defined as two standard deviations above the zero binding) was 0.25 pg/mL and nonspecific binding 2%.

Study Protocol

All subjects abstained from alcohol, caffeine-containing drinks, and food for 12 hours before undergoing the study. Experiments were performed by the same experienced observer in a quiet, temperature-controlled room. In the first phase of the study, baseline measurements were taken over a 30-minute period during saline infusion. Intrabrachial ET-1 (Clinalfa AG, Laufelfingen, Switzerland) was then infused at a constant rate of 5 pmol/min for 90 minutes and recordings of forearm blood flow changes were made at 10-minute intervals.

On a separate occasion, at least 1 week later, again after a 30-minute baseline saline infusion, intrabrachial BQ123 (American Peptide Company, Sunnyvale, CA) was infused at a constant rate of 100 nmol/min over a 90-minute period, with measurements recorded at 10-minute intervals.

Data and Statistics

Data are presented as mean ± SEM. Intergroup demographics and ET-1 concentrations were compared using unpaired *t*-test. Plethysmographic recordings for each subject at each measurement interval were analyzed and group responses were compared by analysis of variance (ANOVA). Group size ($n = 8$), calculated with reference to blood flow data derived from previous measurements of forearm blood flow, provide 90% power to detect a 24% difference at a 5% significance level.[30,31]

Results

Four of the subjects gave a history of borderline hypertension that did not require medical treatment. One control and three patients gave a history of migraine and/or Raynaud's type peripheral circulation. Comparison of demographic data (Table 1) demonstrated that patients with NPG were well matched with control subjects. There were no differences in age, sex, systemic hemodynamic parameters or forearm length between the two groups.

Basal plasma ET-1 concentrations were also similar ($P = 0.81$) in patients (2.39 ± 0.41 pg/mL) and control subjects (3.16 ± 0.54 pg/mL). Systemic arterial blood pressure and heart rate were not altered during infusion of ET-1 (Fig. 1) or BQ123 (Fig. 2), either in patients with NPG or in control subjects.

Infusion of ET-1 reduced forearm blood flow ($P < 0.001$) in patients with NPG and in control subjects (Fig. 3). The magnitude of this response was not altered in patients with NPG ($P = 0.72$; patients versus control subjects). In contrast, infusion of BQ123 increased forearm blood flow ($P < 0.001$) in both groups (Fig. 4). This BQ123-mediated increase in blood flow

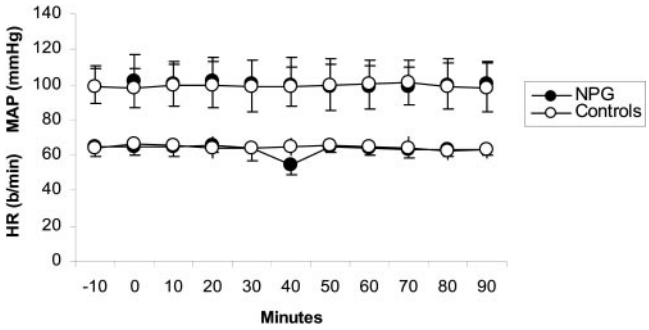

Figure 1. Mean arterial blood pressure and heart rate during ET-1 infusion. NPG and healthy volunteers (Controls) did not differ significantly, and neither group showed significant changes in either parameter during forearm blood flow measurements. Results are expressed as the mean ± SEM and were compared by ANOVA. MAP, mean arterial blood pressure; HR, heart rate; b/min, beats per minute.

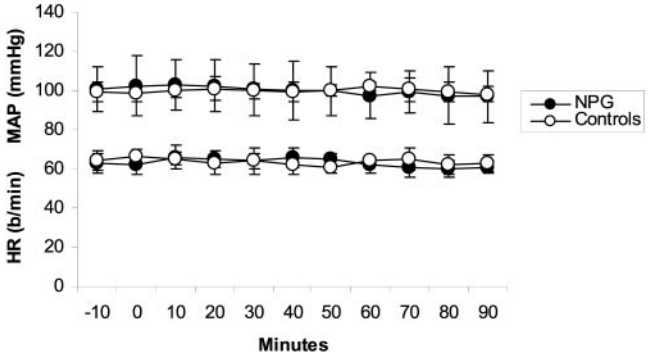

FIGURE 2. Mean arterial blood pressure and heart rate during BQ123 infusion. Patients with NPG and healthy volunteers (Controls) did not differ significantly and neither group showed significant changes in either parameter during forearm blood flow measurements. Results are expressed as the mean ± SEM and were compared using ANOVA. MAP, mean arterial blood pressure; HR, heart rate; b/min, beats per minute.

was attenuated in patients with NPG ($P < 0.001$; patients versus control subjects).

DISCUSSION

Despite normal responses to exogenous ET-1, patients with NPG in our study had an impaired vasodilator response to ET_A receptor antagonism. This finding is consistent with our previous reports of a defect in the peripheral endothelium-derived NO activity in the human forearm[25] and subcutaneous resistance vessels.[26,27] These alterations support the proposition that NPG represents an ocular manifestation of a systemic vascular dysfunction.

Plasma ET-1 Concentrations in Patients with Glaucoma

The hypothesis underlying this investigation was based partly on a previous report of elevated ET-1 concentrations in the plasma of patients with NPG.[21] An increase in circulating ET-1 is associated with endothelial dysfunction and has been reported in a variety of disorders such as congestive heart failure[32] and diabetes.[33] Elevated plasma concentrations of ET-1 may also provide evidence of a common mechanism in the

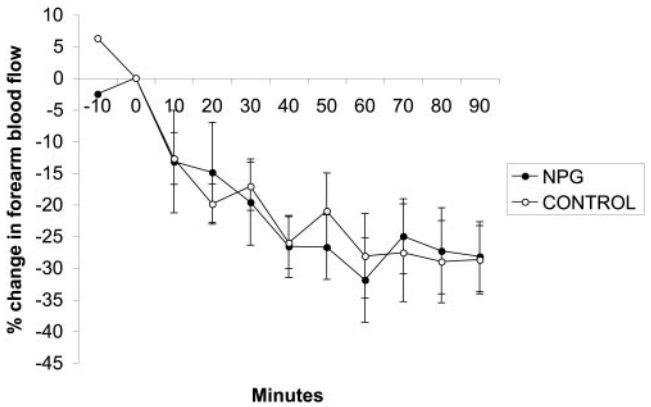

FIGURE 3. Influence of NPG on endothelin-1-mediated reduction of forearm blood flow. Endothelin-1 infusion (5 picomoles/min; 90 minutes) reduced forearm blood flow ($P < 0.001$ for both) to a similar extent in patients with NPG and in matched healthy control subjects ($P = 0.72$). Results are expressed as the mean ± SEM and were compared using ANOVA.

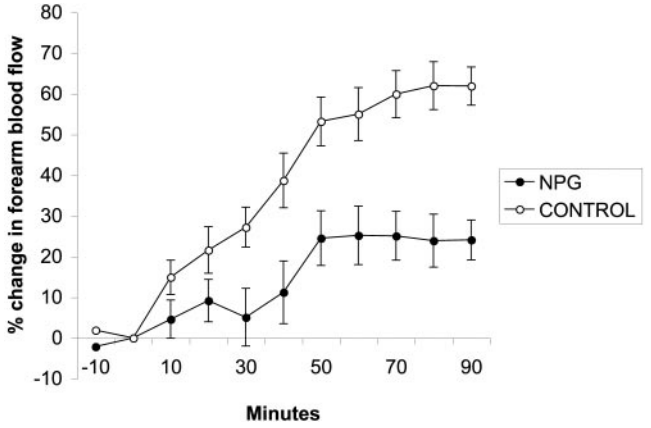

FIGURE 4. Influence of NPG on the increase in forearm blood flow caused by ET_A receptor antagonism. The increase in blood flow ($P < 0.001$ for both) produced by infusion of the ETA antagonist, BQ123, was attenuated in patients with NPG compared with healthy, matched control subjects ($P < 0.001$). Results are expressed as the mean ± SEM and were compared by using ANOVA.

pathogenesis of vasospastic disorders such as Raynaud's phenomenon, migraine, and Prinzmetal's angina.[12] Our results, however, show no alteration in basal plasma ET-1 concentrations in NPG. Although this is at variance with some previous studies,[21] these data are in keeping with others that show no increase in basal ET-1 levels in NPG.[23,24] The results in the study of Nicolela et al.[24] suggest that, analogous to patients with migraine,[34,35] enhanced ET-1 release in patients with NPG is only evident in response to stimuli such as body cooling. This also has some similarities with Raynaud's sufferers in whom there is an exaggerated increase in plasma ET-1 concentrations in response to cold provocation.[20,36] Recently, Emre et al.[37] demonstrated that patients with glaucoma with progressive visual fields had significantly higher plasma ET-1 levels than those with stable fields, despite both groups having controlled IOP.

Notwithstanding these results, it is important to appreciate that the major actions of ET-1 are considered to be autocrine and paracrine (rather than endocrine)[12] and, thus, ET-1 is secreted largely abluminally, toward the vascular smooth muscle.[38] Consequently, plasma concentrations of ET-1 may be difficult to interpret and are at best an indirect measurement of vascular activity of the peptide.

ET-1 Vasoreactivity in NPG

Exogenous ET-1–induced vasoconstriction was not altered by the presence of NPG, suggesting that there is no alteration in smooth muscle sensitivity to ET-1. In contrast, there was an impaired response to ET_A receptor antagonism, suggesting decreased endogenous ET-1-mediated vasoconstriction.

BQ123 is a well-characterized, selective ET_A receptor antagonist. In the presence of ET_A receptor antagonism endogenous ET-1 interacts solely with the ET_B receptor subtype, which is expressed by both endothelial and smooth muscle cells. This produces vasodilatation resulting from reduced ET_A-receptor–mediated vasoconstriction and/or unopposed ET_B-receptor–mediated release of NO from the endothelium.[39] Indeed, we have shown that the vasodilatation induced by ET_A-receptor antagonism in the forearm is predominantly mediated by endothelial NO.[14]

We report that in patients with NPG, there is marked attenuation in the ability of BQ123 to increase forearm blood flow. This could be attributed to lower ET_A-receptor–mediated tone, impaired ET_B-receptor–mediated release of NO, upregulation

of ET_B-receptor–mediated contraction in the vascular smooth muscle, or reduced endogenous ET-1 activity. Of these possibilities, reduced ET-1 activity is unlikely, as plasma ET-1 concentrations were shown to be similar in patients and control subjects. Indeed, the most likely explanation, given the previous demonstration of reduced endothelium-dependent relaxation in this condition, is that ET_B-mediated release of endothelium-derived NO is blunted. This would also be consistent with evidence obtained in isolated resistance arteries that the ability of the endothelium to release vasodilators in response to ET-1 is impaired in patients with NPG.[27] Alternatively, it is possible that the ability of the ET_B receptors in the vascular smooth muscle to induce a compensatory contraction is enhanced by the development of NPG. Against this theory would be the normal basal blood flow and vascular smooth muscle response to ET-1 infusion in this study. Upregulation of ET_B smooth muscle receptors would be unlikely to happen in isolation and if there is an alteration in receptor activity, it is probably more likely to occur in the endothelium.

A Generalized Vascular Defect

In this study, patients with NPG exhibited impaired vasodilatation in response to ET_A-receptor antagonism, probably as a result of altered ET_B-receptor–mediated NO release into the peripheral circulation. This supports our previous studies suggesting that patients with NPG have a generalized vascular dysfunction. It is also possible, however, that these data have direct significance for the ocular circulation. Both ET-1 and NO are active in the eye,[40–46] where they appear to have significant roles in the control of retinal, choroidal and optic nerve head blood flow.[41,43,47–53] Indeed, glaucoma-like optic neuropathy can be induced in animals by using ET-1–mediated, chronic ischemia to the optic nerve head (ONH),[54–56] whereas decreased levels of plasma and aqueous levels of cGMP (an indirect indicator of NO activity) in patients with NPG are associated with reduced flow velocities in the ophthalmic artery.[57] Therefore, impaired ET_B-dependent release of endothelium-derived NO could be expected to have a significant influence on ocular blood flow. An imbalance between NO and ET-1 in the ocular circulation, could render the ONH blood supply more vulnerable either to compression from a "normal" IOP or to transient reductions in ophthalmic blood flow arising from systemic blood pressure dips or provocation from vasospastic stimuli. In addition, ET-1 can contribute to vasospasm by potentiating the responses to other vasoconstrictors such as serotonin and noradrenaline.[12,58] This study suggests, therefore, that a clearer understanding of the alterations in ocular and systemic NO and ET-1 activity is essential for understanding the pathogenesis of and formulating new treatments for NPG.

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