Peripheral Endothelial Dysfunction in Normal Pressure Glaucoma

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Purpose. To assess vascular endothelial function in patients with normal pressure glaucoma using forearm blood flow responses to intra-arterial infusions of endothelial-dependent and -independent vasoactive agents.

Methods. Eight patients with newly diagnosed and untreated normal pressure glaucoma and eight healthy age- and sex-matched control volunteers underwent measurement of forearm blood flow using venous occlusion plethysmography. Blood flow was assessed in response to incremental doses of sodium nitroprusside (an endothelial-independent vasodilator), acetylcholine (an endothelial-dependent vasodilator) and the vasoconstrictor N^G-monomethyl-L-arginine (an inhibitor of nitric oxide synthase).

Results. Sodium nitroprusside caused a dose-related increase in forearm blood flow in patients and controls. Glaucoma patients appeared to have an increased vasodilatory response, but this was not significant ($P = 0.23$). Acetylcholine also induced vasodilatation in both groups, but the response was significantly reduced in the glaucoma group ($P = 0.04$). N^G-monomethyl-L-arginine induced a similar degree of vasoconstriction in both groups ($P = 0.76$).

Conclusions. This study has shown an impairment of peripheral endothelium-mediated vasodilatation in normal pressure glaucoma. These findings would support the concept of a generalized vascular endothelial dysfunction in patients with this condition. (*Invest Ophthalmol Vis Sci.* 1999; 40:1710–1714)

In the absence of an elevation in intraocular pressure, vascular risk factors have been postulated to play a role in normal pressure glaucoma (NPG).[1–8] Vasospastic disorders such as migraine[9,10] and a Raynaud's-like peripheral circulation[11,12] are more prevalent in patients with NPG. Digital blood flow studies have demonstrated an abnormal reaction to cold immersion and delayed cold recovery.[11,12] Vasospasm is characterized by abnormal vascular responsiveness to normal everyday stimuli such as heat and cold.[13,14] Vascular endothelial dysfunction, which is thought to potentiate vasospasm, may therefore also have a role in the pathogenesis of NPG.[15,16]

The vascular endothelium has a vital role in the control of blood flow. In addition to mediating the effects of many hormones and vasoactive agents, it releases factors itself that may act either to contract the vascular smooth muscle, such as endothelin-1,[17] or to relax it, such as nitric oxide (NO).[18,19] Elevated levels of endothelin-1 have been demonstrated in NPG[20] together with abnormal postural responses.[21] Less is known about the role of the NO system in this disease, and it was the purpose of this study to investigate this role.

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Supported by the Royal National Institute for the Blind.

Submitted for publication May 7, 1998; revised November 9, 1998; accepted December 17, 1998.

Proprietary interest category: N.

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Methods

Eight patients with newly diagnosed and untreated NPG were recruited for the study together with eight healthy volunteers matched for age, gender, mean arterial blood pressure, body weight, and forearm length (Table 1). The criteria for the diagnosis of NPG were as follows: mean intraocular pressure of less than 22 mm Hg on diurnal phasing, open anterior chamber angles on gonioscopy, optic disc cupping (with a cup:disc ratio of >0.6) and either thinning or notching of the neural rim, and glaucomatous field loss on the Humphrey perimeter (Humphreys Instruments; Allergan Humphrey, San Leandro, CA) using the 24-2 threshold program (average mean deviation = −7.66 dB and corrected pattern standard deviation = 9.38 dB). Radiology was carried out when indicated to exclude intracranial causes of optic disc anomalies or field loss. None of the patients had a history of previous ocular disease or therapy with steroid medication. All control subjects had a normal ocular examination, intraocular pressure within normal limits, and intact visual fields on automated assessment. Four of the glaucoma patients and one of the healthy volunteers gave a history of either migraine or Raynaud's-type peripheral circulation. Neither group was receiving topical treatment or systemic vasoactive medication, and all were nonsmokers. Approval for the study was granted by the Lothian Research Ethics Committee, and the tenets of the Declaration of Helsinki were observed. Written, informed consent was obtained from each subject before enrollment in the study. All subjects abstained from alcohol, caffeine-containing drinks, and food for 12 hours before the study. The studies were performed by the same experienced operator in a quiet, temperature-controlled room kept at 23.5°C to 24.5°C.

Investigative Ophthalmology & Visual Science, July 1999, Vol. 40, No. 8

TABLE 1. Group Demographics Expressed as Mean (SD)

	NPG (n = 8)	Controls (n = 8)	P
Sex (F:M)	5:3	5:3	
Age (Years)	55.8 (6.8)	58.3 (10.0)	0.56
IOP (mm Hg)	16 (4.0)	14 (1.1)	0.59
Weight (Kg)	69.1 (8.6)	67.6 (5.2)	0.69
MAP (mm Hg)	89.7 (3.9)	90.5 (11.3)	0.82
HR (b/min)	58.6 (8.2)	60.7 (8.4)	0.59
Forearm Length (cm)	24.5 (1.0)	24.6 (0.9)	0.71
Vasospasm	4	1	0.14

IOP, intraocular pressure; MAP (in mm Hg), mean arterial blood pressure; HR, heart rate.

The brachial artery of the nondominant arm was cannulated with a standard wire 27-gauge steel needle (Cooper's Needle Works, Birmingham, UK) attached to a 16-gauge epidural catheter (Portex, Hythe, UK) after subcutaneous injection of 1% lignocaine (Xylocaine; Astra Pharmaceuticals, Kings Langley, UK). Continuous infusion of 0.9% sodium chloride (Baxter Healthcare, Thetford, UK) at a rate of 1 ml/min via an IVAC P1000 syringe pump (IVAC, Basingstoke, UK) maintained patency of the cannula. All drugs were dissolved in physiological saline and administered at a rate of 1 ml/min via the IVAC pump. Two E20 Rapid Cuff Inflators (D. E. Hokanson, WA) were applied to each arm, one at the wrist and one above the elbow. During measurements the lower cuffs were inflated to 220 mm Hg to exclude hand circulation. The upper cuffs were inflated to 35 to 40 mm Hg for 10 seconds in every 15-second interval, achieving venous occlusion and allowing plethysmographic recordings to be made. Forearm blood flow was measured in both arms simultaneously using venous occlusion plethysmography as previously described.[22] The changes in forearm circumference were measured by the mercury-in-silastic strain gauges, placed on the widest part of the forearm, which sensed alterations in forearm circumference that reflected volumetric changes with vasodilation or vasoconstriction. These changes were processed by a MacLab analogue-to-digital converter and Chart version 3.3.8 software (AD Instruments, Castle Hill, Australia) and recorded onto a Macintosh Classic II computer (Apple Computers, Cupertino, CA) calibrated using the internal standard. To reduce the variability of blood flow data, the ratio of flows in the two arms was calculated for each time: in effect using the noninfused arm as a contemporaneous control for the infused arm.[22] Percentage changes in the infused forearm blood flow were calculated as follows[22]:

$$\% \text{ change in blood flow} = 100 \times (I_t/NI_t - I_b/NI_b)/I_b/NI_b$$

where I_b and NI_b are the infused and noninfused forearm blood flows, respectively, at baseline (time 0), and I_t and NI_t are the infused and noninfused forearm blood flows, respectively, 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 (Takeda UA 751; Takeda Medical, Tokyo, Japan).

At the start of each study, baseline measurements were taken over 30 minutes during the infusion of saline. Thereafter, sodium nitroprusside (SNP; David Bull Laboratories, Victoria, Australia) was administered in increasing concentrations of 1, 2, and 4 μg/min for 6 minutes at each dose, and recordings were made for each concentration. Saline was then infused over a 30-minute washout period during which time two measurements were taken, at 10 and 20 minutes. Acetylcholine (ACh) (Miochol; CIBA Vision Ophthalmics, Southampton, UK) was administered in concentrations of 5, 10, and 20 μg/min, and readings were taken after a 6-minute infusion of each concentration. This was again followed by a 30-minute washout with 0.9% saline. Finally, N^G-monomethyl-L-arginine (L-NMMA) (Clinalfa AG, Laufelfingen, Switzerland) at a concentration of 4 μmol/min was infused with measurements taken after 3, 9, and 15 minutes.

The population size (n = 8), based on blood flow data derived from forearm vessel responses, gave a 90% power to detect a 24% difference in blood flow responses at a significance level of 5%.[23,24]

The plethysmographic recordings for each patient at each concentration of the infused agent were analyzed for mean percentage increase or decrease in forearm blood flow. These were grouped together to allow analysis of the mean values for each of the two groups assessed. Group responses were compared using ANOVA.

RESULTS

Sodium nitroprusside induced a dose-related increase in forearm blood flow in both groups ($P < 0.001$ for both). This response appeared higher in the glaucoma group, but this was not significant ($P = 0.23$; Fig. 1A). Acetylcholine also produced a dose-dependent response in both groups ($P < 0.001$ for both). However, the flow increase was significantly lower in the glaucoma group ($P = 0.04$; Fig. 1B). L-NMMA reduced forearm blood flow in both groups. There was no demonstrable difference between the two groups ($P = 0.76$; Fig. 1C). Systemic hemodynamic parameters such as blood pressure and heart rate did not change significantly during the measurements in either group (Fig. 2).

DISCUSSION

This study has shown an impairment of endothelium-mediated vasodilatation, as induced by acetylcholine, in a group of newly diagnosed and untreated patients with NPG. These findings would support the concept of an underlying generalized vascular endothelial dysfunction in these patients.

The high prevalence of vasospastic disorders in NPG patients[8,9,11,12] has led to an increasing interest in the role of the vascular endothelium in this condition. The endothelium is recognized as being an important functional unit in the regulation of blood flow. It forms the inner lining of all blood vessels and therefore lies between the circulating blood and the vascular smooth muscle, acting as both a barrier and modulator of vascular function. It has a diverse number of functions, including control of permeability and the activation and inactivation of hormones. Nitric oxide is synthesized within the endothelial cell from the precursor L-arginine by the action of NO synthase (Fig. 3)[18,19,25] both basally and in response to a variety of stimuli including acetylcholine, histamine, and many other endogenous hormones. Agents such as ACh are

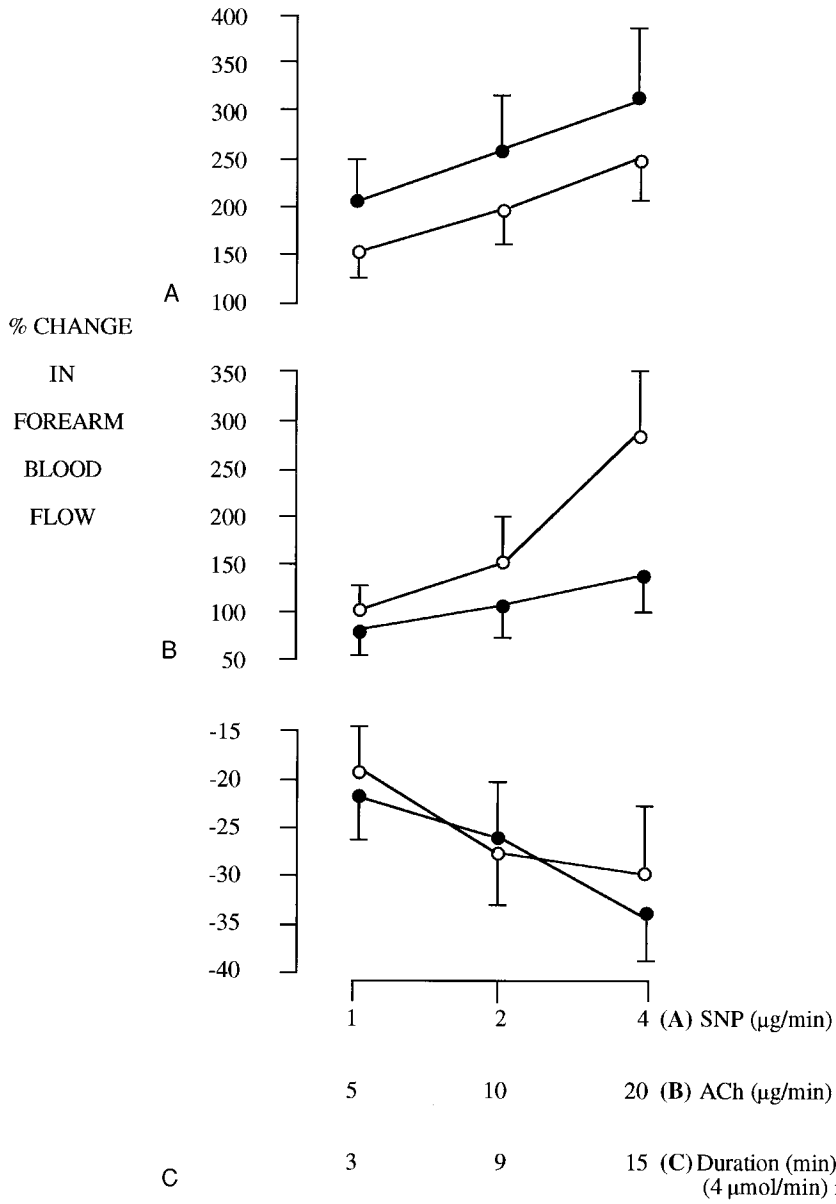

Figure 1. Forearm blood flow responses: mean (SEM). ●, NPG; ○, controls. SNP and Ach are micrograms per minute; L-NMMA is micromoles per minute.

therefore dependent on an intact endothelium to exert their vasodilatory effects.[26] Impairment of a vasodilatory response to acetylcholine suggests endothelial dysfunction, as shown in many other studies.[27–29]

L-NMMA is an analogue of L-arginine and acts as a competitive inhibitor of NO synthase. It reduces the synthesis and release of NO and produces vasoconstriction by withdrawal of endogenous NO-mediated tone in resistance arteries. Therefore, NO bioavailability is indirectly reflected by the reduction in blood flow induced by L-NMMA infusion.[30] Once synthesized and released, NO diffuses to adjacent vascular smooth muscle where it activates guanylate cyclase. Guanylate cyclase increases intracellular levels of cGMP, which acts as a second messenger to induce smooth muscle relaxation. Sodium nitroprusside is an NO donor that has a direct effect on the vascular smooth muscle. The degree of blood flow increase induced by SNP is therefore a measure of endothelium-independent vasodilatation and direct vascular smooth muscle function.

We have assessed vascular endothelial function using the technique of venous occlusion plethysmography, which allows measurement of changes in forearm blood flow in response to intra-arterial infusion of vasoactive agents. This is a well validated technique that has been widely used in the investigation of many vascular diseases,[31] including hypertension,[32,33] heart failure,[34] diabetes mellitus,[35] and Raynaud's phenomenon.[36] By infusing such subsystemic drug concentrations, the vascular effects are localized to the forearm, and no significant systemic hemodynamic changes occur that could confound the vascular responses recorded (Fig. 2).

Although this study has demonstrated a peripheral endothelial dysfunction in patients with NPG, the extent to which this dysfunction may contribute to the glaucomatous process is

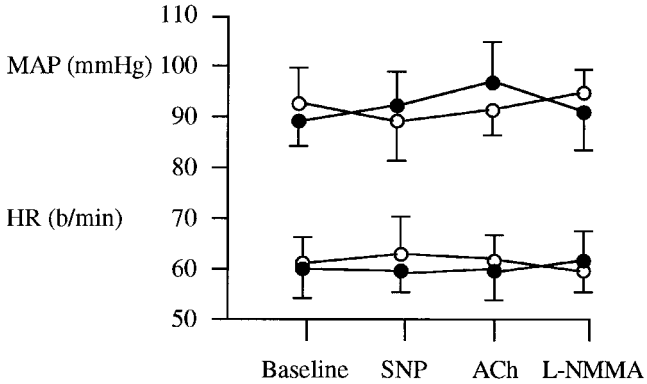

FIGURE 2. Systemic cardiovascular parameters during forearm blood flow measurements. ●, NPG; ○, controls; MAP, mean arterial blood pressure; HR, heart rate.

unclear. The NO system appears to be active in the eye[37,38] and to have a role in both basal and stimulated ocular blood flow in myography studies of isolated human ophthalmic arteries.[39]

Direct evidence for local ocular endothelial dysfunction is difficult to obtain, however. Techniques such as the one described in this article are clearly not suitable for in vivo assessment of the ocular circulation, and other techniques involving intravenous administration of vasoactive agents allow only indirect measurements to be made. In one such study a reduction in the pulsatile component of choroidal blood flow resulted from intravenous infusion of L-NMMA in healthy volunteers.[40] Whether this resulted from local inhibition of NO synthase in the ocular circulation or from changes in systemic factors such as blood pressure and cardiac output is difficult to ascertain.

In conclusion, therefore, this study has demonstrated evidence of generalized endothelial dysfunction in a group of untreated patients with NPG. Whether this dysfunction exists in the ophthalmic circulation, and whether it contributes to the glaucomatous process, remains to be determined.

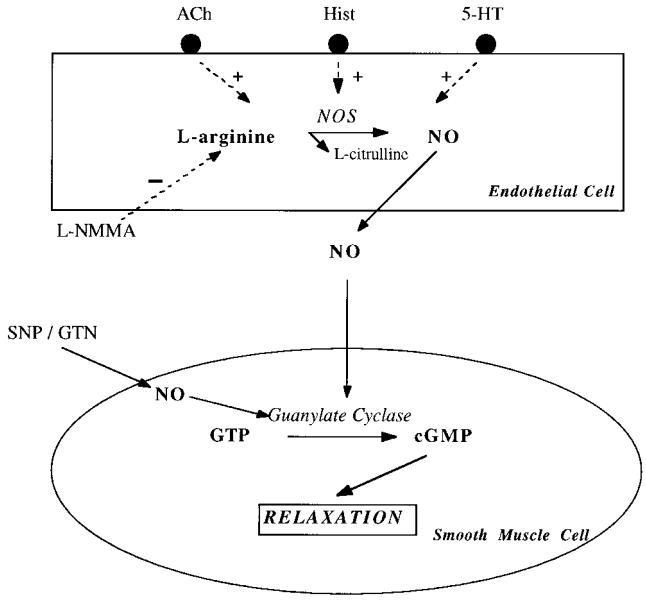

FIGURE 3. Endothelial-derived NO synthesis and activity. Hist, histamine; 5-HT, serotonin; NOS, NO synthase; GTN, glycerol trinitrate; GTP, guanylate triphosphate; cGMP, cyclic guanylate monophosphate.

References

1. Feldman F, Sweeney V, Drance S. Cerebro-vascular studies in chronic simple glaucoma. *Can J Ophthalmol.* 1969;4:358–364.
2. Drance SM. Some factors in the production of low tension glaucoma. *Br J Ophthalmol.* 1972;56:229–242.
3. Levene R. Low tension glaucoma: a critical review and new material. *Surv Ophthalmol.* 1980;24:621–664.
4. Carter CJ, Brooks DE, Doyle DL, Drance SM. Investigations into a vascular aetiology for low-tension glaucoma. *Ophthalmology.* 1989;97:49–55.
5. Flammer J. The vascular concept of glaucoma. *Surv Ophthalmol.* 1994;38(suppl):SS3–S6.
6. Hitchings R. Low tension glaucoma—its place in modern glaucoma practice. *Br J Ophthalmol.* 1992;76:494–496.
7. Sonnsjo B, Krakau CE. Arguments for a vascular glaucoma etiology. *Acta Ophthalmol.* 1993;71:433–444.
8. Broadway D, Drance S. Glaucoma and vasospasm. *Br J Ophthalmol.* 1998;82:862–870.
9. Phelps CD, Corbett JJ. Migraine and low-tension glaucoma. *Invest Ophthalmol Vis Sci.* 1985;26:1105–1108.
10. Klein B, Klein R, Meuer S, Goetz L. Migraine headache and its association with open angle glaucoma: the Beaver Dam study. *Invest Ophthalmol Vis Sci.* 1993;34:3024–3028.
11. Drance SM, Douglas GR, Wijsman K, Schulzer M, Britton RJ. Response of blood flow to warm and cold in normal and low-tension glaucoma patients. *Am J Ophthalmol.* 1988;105:35–39.
12. Gasser P, Flammer J. Blood cell velocity in the nailfold capillaries of patients with normal tension and high tension glaucoma. *Am J Ophthalmol.* 1991;111:585–588.
13. Dowd P. Raynaud's phenomenon. *Lancet.* 1995;346:283–290.
14. Walmsley D, Goodfield MJD. Evidence for an abnormal peripherally mediated vascular response to temperature in Raynaud's phenomenon. *Br J Rheumatol.* 1990;29:181–184.
15. Flammer J. Do ocular vasospasms help cause low tension glaucoma? *Doc Ophthalmol Proc Ser.* 1987;49:397–399.
16. Guthauser U, Flammer J, Mahler F. The relationship between digital and ocular vasospasm. *Graefes Arch Clin Ophthalmol.* 1988;226:224–226.
17. Webb DJ, Haynes WG. Endothelins come of age. *Lancet.* 1993; 342:1439.
18. Palmer R, Ashton D, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature.* 1988;333:664–666.
19. Moncada S, Higgs A. The L-arginine—nitric oxide pathway. *N Engl J Med.* 1993;329:2002–2012.
20. Sugiyama T, Moriya S, Oku H, Azuma I. Association of endothelin-1 with normal tension glaucoma: clinical and fundamental studies. *Surv Ophthalmol.* 1995;39(suppl 1):S49–S56.
21. Kaiser HJ, Flammer J, Wenk M, Luscher T. Endothelin-1 plasma levels in normal tension glaucoma; abnormal response to postural changes. *Graefes Arch Clin Exp Ophthalmol.* 1995;233:484–488.
22. Benjamin N, Calver A, Collier J, Robinson B, Vallance P, Webb D. Measuring forearm blood flow and interpreting the responses to drugs and mediators. *Hypertension.* 1995;25:918–923.
23. Newby D. Intra-arterial substance P mediated vasodilation in the human forearm: pharmacology, reproducibility and tolerability. *Br J Pharmacol.* 1997;43:493–499.
24. Newby D, Flint L, Fox K, Boon N, Webb D. Reduced responsiveness to endothelin-1 in peripheral resistance vessels of patients with Syndrome X. *J Am Coll Cardiol.* 1998;31:1585–1590.
25. Palmer RMJ. The L-arginine: nitric oxide pathway. *Curr Opin Nephrol Hypertens.* 1993;2:122–128.
26. Furchgott R, Zawadski J. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature.* 1980;299:373–547.
27. Chowienczyk P, Watts G, Cockcroft J, Ritter J. Impaired endothelium-dependent vasodilation of forearm resistance vessels in hypercholesterolaemia. *Lancet.* 1992;340:1430–1432.
28. Stoes E, Koomans H, de Bruin T, Rabelink T. Vascular function in the forearm of hypercholesterolaemic patients off and on lipid-lowering medication. *Lancet.* 1995;346:467–471.

29. Heizer T, Yis–Herthuala S, Luoma J, et al. Cigarette smoking potentiates dysfunction of forearm resistance vessels in patients with hypercholesterolaemia: role of oxidized LDL. *Circulation.* 1996; 93:1346–1353.
30. Noon J, Haynes W, Webb D, Shore A. Local inhibition of nitric oxide generation in man reduces blood flow in the finger pulp but not hand dorsum skin. *J Physiol.* 1996;49:501–508.
31. Wennmalm A. Endothelial nitric oxide and cardiovascular disease. *J Intern Med.* 1994;235:317–327.
32. Panza J, Quyyumi A, Brush J, Epstein S. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med.* 1990;323:22–27.
33. Panza J, Kilcoyne C, Quyyumi A. The role of endothelium-derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. *Circulation.* 1993;87:1468–1474.
34. Hirooka Y, Imaizuma T, Tagawa T, Shiramoto M, Endo T, Ando S. Effects of L-arginine on impaired acetylcholine-induced and ischemic vasodilation of the forearm in patients with heart failure. *Circulation.* 1994;90:658–668.
35. Calver A, Collier J, Vallance P. Inhibition and stimulation of nitric oxide synthesis in the human forearm arterial bed of patients with insulin-dependent diabetes. *J Clin Invest.* 1992;90:2548–2554.
36. Brain S, Petty R, Lewis J, Williams T. The effect of intradermal CGRP and histamine on blood flow in the forearm of normal volunteers and Raynaud's sufferers. *Br J Clin Pharmacol.* 1989; 28:736–737.
37. Becquet F, Courtois Y, Goureau O. Nitric oxide in the eye: multifaceted roles and diverse outcomes. *Surv Ophthalmol.* 1997;42: 71–81.
38. Meyer P, Flammer J, Luscher T. Endothelium-dependent regulation of the ophthalmic microcirculation in the perfused porcine eye: role of nitric oxide and endothelins. *Invest Ophthalmol Vis Sci.* 1993;34:3614–3621.
39. Haefliger I, Flammer J, Luscher T. Nitric oxide and endothelin-1 are important regulators of human ophthalmic artery. *Invest Ophthalmol Vis Sci.* 1992;33:2340–2343.
40. Schmetterer L, Krejcy K, Kastner J, et al. The effect of systemic nitric oxide synthase inhibition on ocular fundus pulsations in man. *Exp Eye Res.* 1997;64:305–312.