Nitric Oxide and Ocular Blood Flow in Patients With IDDM

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Endothelial dysfunction has been implicated in the pathogenesis of diabetic vascular disorders such as diabetic retinopathy. We hypothesized that either local endogenous nitric oxide (NO) synthesis or local reactivity to endogenous NO might be impaired in patients with IDDM and that this may contribute to the development of diabetic retinopathy. Ten otherwise healthy patients with long-standing IDDM and ten healthy control subjects were studied according to an open randomized two-way cross-over design. Subjects received intravenous infusions of either N^G-monomethyl-L-arginine, an inhibitor of NO-synthase, or L-arginine, the precursor of NO synthesis, on two separate study days. Ocular hemodynamics were assessed by laser interferometric measurement of fundus pulsations and Doppler sonographic measurement of blood flow velocity in the ophthalmic artery. N^G-monomethyl-L-arginine decreased fundus pulsations and blood flow velocity in the ophthalmic artery and increased blood pressure in healthy subjects. The responses to NO-synthase inhibition were significantly less in diabetic subjects. In contrast, L-arginine caused a comparable increase in fundus pulsations and decrease in blood pressure in both cohorts. These results indicate that systemic and ocular hemodynamic reactivity to NO-synthase inhibition is reduced in patients with long-standing IDDM, compared with healthy control subjects. Thus, this study indicates that either NO-synthase activity is increased or NO sensitivity is decreased in patients with IDDM and supports the concept of an involvement of the L-arginine–NO system in the pathophysiology of diabetic retinopathy. *Diabetes* 46:653–658, 1997

Diabetes is associated with increased mortality and morbidity due to vascular complications (1). Endothelial dysfunction has been implicated in the pathogenesis of diabetic long-term complications. There is evidence from in vitro and in vivo studies that nitric oxide (NO)-mediated vasodilation may be impaired in diabetes (1–5). NO, which is synthesized by the vascular endothelium from L-arginine, is a potent vasodilator (6) and contributes to the regulation of vascular tone in humans (7).

Endothelium-derived NO has also been shown to regulate ocular circulation. In isolated human ophthalmic arteries, NO is a very potent modulator of vascular tone (8), and systemic NO-synthase inhibition significantly decreases choroidal blood flow in animals (9,10) and humans (11). Although endothelial dysfunction is assumed to contribute to altered ophthalmic circulation and the development of diabetic retinopathy (12), there are no experimental data confirming this hypothesis.

Based on these observations, we hypothesized that either local NO synthesis or local reactivity to endogenous NO might be impaired in patients with IDDM and that this may contribute to the development of diabetic retinopathy. We have therefore compared the effects of systemic NO-synthase inhibition by N^G-monomethyl-L-arginine (L-NMMA) and of L-arginine on ocular hemodynamics in patients with long-standing IDDM and in healthy subjects. Fundus pulsations were measured by laser interferometry (13), and blood flow velocities in the ophthalmic artery were measured with Doppler imaging (14). Laser interferometric measurement of fundus pulsations has been shown to estimate pulsatile choroidal blood flow with high reproducibility (15). Exhaled NO and plasma nitrates were measured as surrogate markers of endogenous NO production.

RESEARCH DESIGN AND METHODS

Subjects. After approval from the Ethics Committee of Vienna University School of Medicine, 10 male subjects with IDDM (age: mean ± SD, 35.2 ± 10.2 years; range, 25–59 years) and 10 age-matched healthy male volunteers were studied (age: mean ± SD, 34.6 ± 11.0 years; range, 24–61 years). The nature of the study was explained, and all subjects gave written consent to participate. Each subject passed a screening examination that included medical history and physical examination, 12-lead electrocardiogram, complete blood count, activated partial thromboplastin time, thrombin time, clinical chemistry (sodium, potassium, creatinine, uric acid, glucose, cholesterol, triglycerides, alanine aminotransferase, aspartate transcarbamylase, γ-glutamyltransferase, alkaline phosphatase, total bilirubin, and total protein), hepatitis A, B, C, and HIV serology, urine analysis, and a urine drug screening. Subjects were excluded if any abnormality was found as part of the pretreatment screening, unless the investigators considered an abnormality to be clinically irrelevant or caused by diabetes.

Inclusion criteria in the diabetes study group were IDDM for >10 years, HbA_{1c} <8.5%, and microalbuminuria <100 μg/min. Diagnosis of IDDM was defined by a history of sudden ketoacidotic onset of the disease and a missing or markedly impaired stimulated C-peptide concentration (<1.0 ng/ml) following intravenous arginine administration. Exclusion criteria were moderate or severe systemic hypertension (defined as systolic blood pressure >165 mmHg and diastolic blood pressure >100 mmHg) or coronary heart disease as evidenced from prestudy bicycle exercise test. Cardiac autonomic neuropa-

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Received for publication 24 June 1996 and accepted in revised form 8 November 1996.

ANOVA, analysis of variance; FPA, fundus pulsation amplitude; L-NAME, N^G-nitro-L-arginine methyl ester; L-NMMA, N^G-monomethyl-L-arginine; MAP, mean arterial pressure; MFV, mean flow velocity; OA, ophthalmic artery; PR, pulse rate; STZ, streptozotocin.

TABLE 1
Characteristics of subjects with IDDM

Subject	Age (years)	Diabetes duration (years)	Retinopathy	HbA_{1c} (%)	Microalbuminuria (μg/min)
1	34	20	Level 3	6.3	12.7
2	39	12	Level 1	6.7	6.4
3	42	17	Level 3	6.6	6.3
4	38	16	Level 1	7.6	22.4
5	59	35	Level 2	7.2	27.0
6	31	19	Level 1	8.1	2.4
7	25	12	Level 1	8.1	5.6
8	29	15	Level 2	8.4	20.3
9	25	17	Level 1	8.5	13.1
10	30	28	Level 3	7.1	8.8

Stages of retinopathy were defined according to Modified Airlie House Classification (16,17). Level 1, no retinopathy; level 2, microaneurysms (one or more) only; level 3, microaneurysms and one or more of the following: retinal hemorrhages, but total hemorrhages and microaneurysms less than Standard Photo #2; hard exudates less than Standard Photo #3; soft exudates questionably present; intraretinal microvascular abnormalities questionably present, venous beading questionably present, or venous focal narrowing or loops definitely present.

thy was excluded by heart rate-variability measurements during rest, Valsalva maneuver, deep respiration, and orthostasis (Ewing test).

Subjects' right eyes were studied. Both study groups underwent an ophthalmic examination including slit lamp biomicroscopy and indirect funduscopy. In the diabetic group, additional color fundus photography of seven fields and fluorescence angiography was performed, and the eyes were classified according to the Modified Airlie House Classification (16,17). Subjects were excluded if any ocular disease, except diabetic retinopathy, was evidenced at the prestudy ophthalmic examination, when visual acuity was <100%, or when refractive error was >3 diopters.

Characteristics of the diabetic patients are given in Table 1. All patients were in good diabetic control without microalbuminuria. All patients were treated by an intensified insulin therapy, using a multiple daily insulin injection program after they had participated in a structured diabetes education and care program (18). None of the subjects had blood pressures >140/80 mmHg, and none of the subjects took any medication besides insulin.

Study design. Subjects were studied according to an open randomized two-way cross-over design with washout periods of at least 7 days. Subjects abstained from alcohol and stimulating beverages containing xanthine derivatives 12 h before drug administration. On trial days, subjects received their regular and intermediate insulin preparation and arrived after a light breakfast.

L-NMMA day. After a 20-min resting period in a sitting position, baseline measurements of Doppler sonography, laser interferometry, exhaled NO, and blood pressure were taken, and a venous blood sample was collected. Thereafter L-NMMA (3 mg/kg) was infused over 5 min. Hemodynamic measurements, measurement of exhaled NO, and blood sampling were repeated at 15, 35, and 45 min after the start of L-NMMA infusion.

L-arginine day. After a 20-min resting period in a sitting position, baseline measurements of ocular hemodynamic parameters, exhaled NO, and blood pressure were taken, and a venous blood sample was collected. Thereafter L-arginine was administered intravenously over 30 min (420 mg/kg). Noninvasive hemodynamic measurements, measurement of exhaled NO, and blood sampling were done at baseline and 20 and 50 min after the start of infusion.

Measurements were performed in a predetermined order (Doppler sonography of the ophthalmic artery, fundus pulsation measurements, blood pressure and pulse rate, blood sampling, and exhaled NO). Pulse rate and a real-time electrocardiogram were monitored continuously during the study period.

The time points of measurements after L-NMMA administration were selected according to the results of our previous studies, where the maximum effect on fundus pulsations was observed 15–45 min after the start of L-NMMA bolus infusion (11). The pharmacodynamic effects of L-arginine were estimated on the basis of previous studies, which indicate that the maximum effect of L-arginine is observed at the end of the infusion period (19,20). Thus, the design of the study was not blinded because of the different infusion periods for L-arginine and L-NMMA and the different time points of measurements. To minimize observer bias, the evaluation of the Doppler ultrasound and the fundus pulsation measurements were observer blinded.

Methods

Fundus pulsations. Pulse synchronous pulsations of the ocular fundus were assessed by laser interferometry on the subject's right eye. The method is described in detail by Schmetterer et al. (13). Briefly, the eye is illuminated by the beam of a single-mode laser diode with a wavelength (λ) of 783 nm. The light is reflected at both the front side of the cornea and the retina. The two re-emitted waves produce interference fringes from which the distance changes between cornea and retina during a cardiac cycle can be calculated. Distance changes between cornea and retina lead to a corresponding variation of the interference order [$\Delta N(t)$]. This change in interference order can be evaluated by counting the fringes moving inwards and outwards during the cardiac cycle. Changes in optical distance [$\Delta L(t)$], corresponding to the cornea-retina distance changes, can then be calculated by $\Delta L(t) = \Delta N(t) \cdot \lambda/2$. The maximum distance change is called fundus pulsation amplitude (FPA) and estimates the local pulsatile blood flow in the selected ocular vessels (15). The short-term and day-to-day variability of the method is small, which allows the detection of even small changes in local pulsatile blood flow after pharmacological stimulation (11,13,15,21). To obtain information on the choroidal blood flow, the macula, where the retina lacks vasculature, was chosen for measurements.

Doppler sonography. Blood flow velocities in the right ophthalmic artery (OA) were assessed using Doppler ultrasound (14). The OA was measured anteriorly, at the point where it crosses the optic nerve, with a 7.5-MHz probe (CFM 750, Vingmed Sound, Horten, Norway). Mean flow velocity (MFV) was calculated as time mean of the spectral outline.

Measurement of exhaled NO. Exhaled NO was measured with a chemoluminescence detector (nitrogen oxides analyzer, Model 8840, Monitor Labs) connected to a strip-chart recorder. Calibration of the instrument was done with certified gases (300 ppb NO in N_2, AGA, Vienna, Austria) by precision flow meters. A baseline signal was obtained with pure nitrogen. A total of 1,000 ml/min of the exhaled air was allowed to enter the inlet port. Subjects were instructed to fully inflate their lungs, hold their breath for 10 s, and exhale for 10 s into a teflon tube. Three consecutive readings were made at each measurement point under nasal occlusion. The end-expiratory values from the strip recorder readings were used for analysis. This assures that inspired NO from the ambient air does not distort the results (22). This method of quantifying the degree of endogenous NO synthesis has already been used previously (23,24).

Plasma levels and microalbuminuria. Plasma nitrate concentrations were determined colorimetrically after reaction with the Griess reagent, as described in detail by Roth et al. (25). Insulin, glucose, and C-peptide concentrations were measured by routine procedures. Microalbuminuria was expressed as albumin excretion rate (μg/min). Albumin was measured by turbidimetry on Hitachi 717 analyzer (Boehringer Mannheim, Mannheim, Germany) from a 24-h urine sample.

Noninvasive measurement of systemic hemodynamics. Systolic (sBP) and diastolic (dBP) blood pressure were measured on the upper arm by an automated oscillometric device. Mean arterial pressure (MAP) was calculated as 1/3 sBP + 2/3 dBP. Pulse rate (PR) was automatically recorded from a finger pulse-oxymetric device, and electrocardiogram was taken from a standard device (HP-CMS patient monitor, Hewlett Packard, Palo Alto, CA).

Data analyses. For data description, hemodynamic parameters and exhaled NO levels were expressed as a percentage change from baseline (Δ%). Effects

TABLE 2
Baseline values of the two study days

	Day 1	Day 2
Healthy subjects (n = 10)		
FPA (μm)	4.4 ± 1.2	4.4 ± 1.1
MFV in the OA (cm/s)	20.3 ± 1.2	18.5 ± 1.3
MAP (mmHg)	81.8 ± 2.2	85.1 ± 2.7
PR (min^{-1})	73.2 ± 2.9	74.2 ± 3.9
Exhaled NO (ppb)	51.4 ± 8.5	55.0 ± 10.1
Diabetic subjects (n = 10)		
FPA (μm)	4.1 ± 0.4	4.1 ± 0.4
MFV in the OA (cm/s)	23.4 ± 2.4	19.5 ± 1.3
MAP (mmHg)	80.8 ± 2.0	82.9 ± 1.3
PR (min^{-1})	71.7 ± 3.3	75.4 ± 3.9
Exhaled NO (ppb)	37.4 ± 6.3	37.6 ± 8.6

Data are means ± SE. Washout period between the two study days was at least 7 days.

of L-arginine and L-NMMA on hemodynamic parameters and differences between study groups were assessed by repeated measure analysis of variance (ANOVA). Post hoc comparisons were done with paired t test at individual time points. A P value <0.05 was considered significant. For data description, values are given as means ± SE.

RESULTS

Baseline values of the two study days are given in Table 2. No significant differences between the two study days were observed. Moreover, there were no significant differences in the measured parameters between the two study groups. Exhaled NO was 30% lower in patients with diabetes, compared with control subjects, but this difference did not reach the level of significance because of the high interindividual variability.

L-NMMA significantly decreased FPA versus baseline in both study groups (–8% in IDDM patients, $P < 0.05$; –18% in control subjects, $P < 0.005$; Fig. 1; Table 3). The effect on FPA was significantly smaller in diabetic patients, compared with healthy subjects ($P < 0.05$ between groups). MFV in the OA was significantly reduced in the control group (–17%, $P < 0.05$), but not in the diabetic group (–10%, NS) versus baseline. However, the effect on MFV in the OA was only significantly different between the two study groups 30 min after L-NMMA administration as evidenced by post hoc comparisons ($P < 0.05$). MAP increased after L-NMMA in healthy subjects (8%, $P < 0.05$) but not in patients with diabetes (2%, NS); the response was significantly different between the two study groups ($P < 0.05$). Exhaled NO decreased in both groups after systemic NO-synthase inhibition versus baseline (45% in IDDM patients and 55% in control subjects, $P < 0.005$). No significant changes were observed in plasma nitrates, insulin, C-peptide, or glucose following L-NMMA administration (Table 4).

L-arginine significantly increased FPA in IDDM patients and control subjects versus baseline (10% in IDDM patients and 11% in control subjects, $P < 0.05$ in both groups; Fig. 2; Table 5), whereas MFV in the OA was unchanged. A small but significant decrease in MAP was observed in both groups (–4% in IDDM patients and –7% in control subjects versus baseline, $P < 0.05$). There were significant increases versus baseline in exhaled NO (25% in IDDM patients and 20% in control subjects, $P < 0.05$ versus baseline; Fig. 2) and in plasma nitrate levels (35% in IDDM patients and 21% in control subjects, $P < 0.05$ versus baseline; Table 6). L-arginine tended to increase insulin and C-peptide plasma levels, which, however, did not reach statistical significance (Table 6). Glucose was not affected by L-arginine. There were no differences between IDDM patients and control subjects for any of the measured variables in response to L-arginine.

DISCUSSION

The main finding of the present study is that both the systemic and ocular hemodynamic responses to NO-synthase inhibition are reduced in patients with IDDM, compared with age-matched healthy subjects. This is in agreement with findings from previous animal studies: a diminished vasoconstrictor response to systemic NO-synthase inhibition by N^G-nitro-L-arginine methyl ester (L-NAME) has already been observed in rats with streptozotocin (STZ)-induced diabetes (2). Also, the effect of intradermal L-NAME administration on skin blood flow in STZ-induced diabetic rats is reduced (4). Moreover, in STZ-induced diabetic rats, systemic and renal hemo-

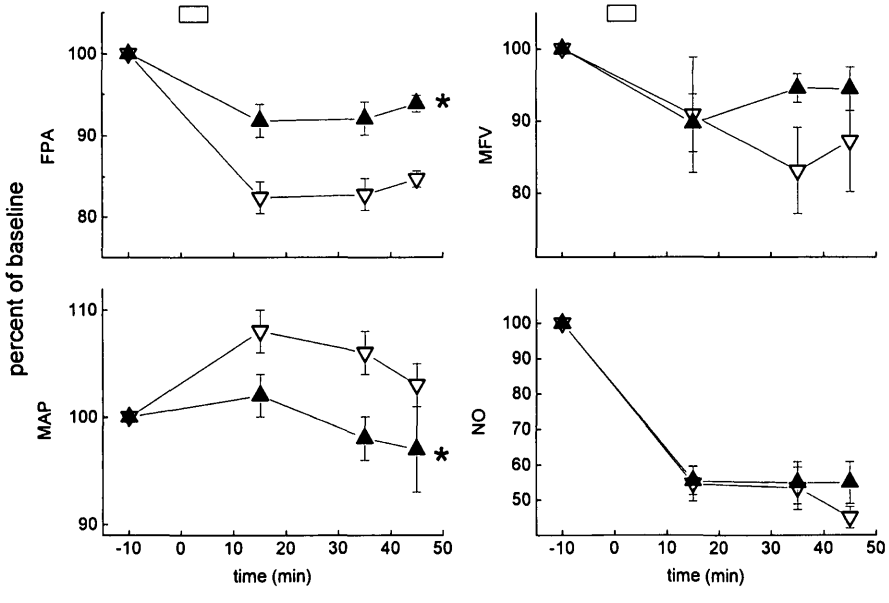

FIG. 1. Time course of the effect of systemic infusion of L-NMMA (3 mg/kg) in patients with IDDM (n = 10, ▲) and healthy control subjects (n = 10, ▽) on FPA, MAP, MFV in the ophthalmic artery, and exhaled NO. L-NMMA infusion is indicated by a box. The data are expressed as percentage of baseline. Error bars represent the standard error of the means. *Significant differences between study groups as calculated by repeated measure ANOVA ($P < 0.05$).

TABLE 3
The effects of L-NMMA on FPA, MFV in the OA, MAP, and exhaled NO

	Baseline	15 min	35 min	45 min
Healthy subjects (n = 10)				
FPA (μm)	4.4 ± 1.3	3.6 ± 1.1	3.6 ± 1.2	3.7 ± 1.3*
MFV (cm/s)	19.6 ± 1.3	17.8 ± 1.1	16.3 ± 1.3	17.1 ± 1.2*
MAP (mm Hg)	84.2 ± 2.3	90.9 ± 2.7	89.3 ± 2.4	86.7 ± 2.5*
Exhaled NO (ppb)	53.0 ± 10.4	29.0 ± 6.3	28.3 ± 6.9	23.9 ± 6.0*
Diabetic subjects (n = 10)				
FPA (μm)	4.1 ± 0.4	3.8 ± 0.5	3.8 ± 0.4	3.9 ± 0.4*
MFV (cm/s)	21.4 ± 1.8	19.2 ± 1.7	20.2 ± 1.5	20.2 ± 1.7
MAP (mm Hg)	80.5 ± 1.4	82.1 ± 1.3	78.9 ± 1.3	78.1 ± 1.3
Exhaled NO (ppb)	38.5 ± 7.6	21.4 ± 5.2	21.1 ± 4.7	21.2 ± 5.1*

Data are means ± SE. *Significant changes versus baseline following L-NMMA administration as calculated by ANOVA.

dynamic responses to systemic NO synthase blockade with L-NAME are blunted (3). In humans, impaired vasoconstriction after local infusion of L-NMMA into the brachial artery was observed in patients with IDDM (1) and NIDDM (5).

In contrast to the different response to L-NMMA, the effect of stimulation of NO synthesis by L-arginine were almost identical in diabetic patients and control subjects. L-arginine caused a significant decrease in MAP and a significant increase in FPA. A blood pressure effect following systemic L-arginine administration has already been reported previously in healthy subjects (19,20,26) and in patients with IDDM (26). It cannot entirely be excluded that L-arginine acts via additional other mechanisms than stimulation of NO synthesis (26). In our study, plasma insulin levels tended to increase (Table 4), which is in agreement with previous findings (27,28) and could contribute to the vasodilating effects of L-arginine (29). However, the administration of L-arginine caused a ~20% increase in exhaled NO in both study groups, which is in keeping with previous findings in healthy subjects (22). Hence, we propose that the observed hemodynamic effects are at least in part mediated by an increase in endogenous NO production. This is further supported by the observed increase in plasma nitrate levels.

Our results most likely reflect an increased basal NO-synthase activity in IDDM patients, compared with control subjects. Therefore, the blunted response to L-NMMA in IDDM patients is consistent with the impaired inhibition of NO-synthase. The larger increase in MAP in control subjects than in IDDM patients argues that a higher dose of L-NMMA would be necessary to achieve the same effect in IDDM patients. The less pronounced response in FPA in diabetic subjects again argues in favor of reduced vasoconstriction after L-NMMA administration. This interpretation is in keeping with previous findings on the effect of aminoguanidine, an inhibitor of NO-synthase, on MAP in diabetic and control rats (30–32). Moreover, NO-synthase inhibitors delay the onset of diabetes in spontaneously diabetic rats, which provides another line of evidence for increased NO production in diabetes (33).

On the other hand, it might be argued that baseline levels in exhaled NO were ~30% lower in IDDM patients than in control subjects. However, the exact origin of NO in exhaled air has not yet been identified. Although recent studies indicate that in normal subjects exhaled NO is derived from constitutive forms of NO synthase (34), it is not clear whether interindividual differences in exhaled NO levels can be used to draw conclusions on NO-synthase activity.

Our results during both L-NMMA and L-arginine administration would also be compatible with a shift of the NO-production dose-response curve to the right in patients with diabetes. Since the vasodilation during L-arginine administration was similar in the two study groups, the maximum response to stimulated endogenous NO production seems to be unaltered in patients with diabetes. With the dose of L-arginine that we chose for the present study, the synthesis of NO

TABLE 4
The effects of L-NMMA on plasma nitrate, glucose, insulin, and C-peptide levels

	Baseline	15 min	35 min	45 min
Healthy subjects (n = 10)				
Nitrates (μmol/l)	26.3 ± 5.2	30.2 ± 9.1	27.5 ± 6.6	35.5 ± 14.0
Insulin (μU/ml)	8.0 ± 1.9	5.8 ± 1.1	12.2 ± 5.1	11.2 ± 2.0
Glucose (mg/100 ml)	82.2 ± 5.4	79.0 ± 4.1	78.6 ± 3.3	80.6 ± 3.6
C-peptide (ng/ml)	1.89 ± 0.34*	1.58 ± 0.21	1.71 ± 0.23	1.89 ± 0.25
Diabetic subjects (n = 10)				
Nitrates (μmol/l)	23.1 ± 4.4	24.0 ± 3.6	24.6 ± 4.4	22.7 ± 3.6
Insulin (μU/ml)	15.5 ± 3.9	18.6 ± 4.1	17.3 ± 3.9	16.3 ± 5.1
Glucose (mg/100 ml)	144.8 ± 15.1	146.3 ± 16.5	142.6 ± 15.7	143.9 ± 15.0
C-peptide (ng/ml)	0.06 ± 0.03*	0.05 ± 0.02	0.07 ± 0.03	0.09 ± 0.05

Data are means ± SE. *Significant differences between baseline values of healthy and diabetic subjects.

TABLE 5
The effect of L-arginine on fundus pulsation amplitude (FPA), mean flow velocity in the OA (MFV), mean blood pressure (MAP), and exhaled NO (NO)

	Baseline	20 min	50 min
Healthy subjects (n = 10)			
FPA (μm)	4.5 ± 1.1	5.0 ± 1.1	4.9 ± 1.2*
MFV (cm/s)	19.2 ± 1.3	20.0 ± 1.5	18.2 ± 1.2
MAP (mmHg)	82.7 ± 2.6	76.9 ± 2.4	75.3 ± 2.3*
NO (ppb)	53.4 ± 9.8	64.1 ± 10.3	58.7 ± 9.3*
Diabetic subjects (n = 10)			
FPA (μm)	4.0 ± 0.5	4.4 ± 0.6	4.3 ± 0.7*
MFV (cm/s)	21.5 ± 1.3	21.7 ± 1.3	22.1 ± 1.5
MAP (mmHg)	83.2 ± 1.5	79.9 ± 1.4	79.0 ± 1.3*
NO (ppb)	36.5 ± 7.3	45.3 ± 7.1	45.6 ± 8.1*

Data are means ± SE. *Significant changes versus baseline following L-arginine administration as calculated by ANOVA.

TABLE 6
The effect of L-arginine on plasma nitrate, glucose, insulin, and C-peptide levels

	Baseline	20 min	50 min
Healthy subjects (n = 10)			
Nitrates (μmol/l)	27.8 ± 8.3	30.6 ± 5.6	33.5 ± 9.4†
Insulin (μU/ml)	10.5 ± 3.2	13.0 ± 7.2	12.3 ± 3.8
Glucose (mg/100 ml)	84.9 ± 2.7	90.7 ± 3.5	81.0 ± 4.9
C-peptide (ng/ml)	1.96 ± 0.39*	3.93 ± 0.51	3.42 ± 0.50
Diabetic subjects (n = 10)			
Nitrates (μmol/l)	23.1 ± 4.7	28.4 ± 4.9	31.1 ± 7.0†
Insulin (μU/ml)	16.8 ± 13.4	20.8 ± 4.1	22.5 ± 7.6
Glucose (mg/100 ml)	138.5 ± 31.3	166.5 ± 30.7	170.6 ± 32.1
C-peptide (ng/ml)	0.11 ± 0.06*	0.16 ± 0.09	0.18 ± 0.10

*Significant differences between baseline values of healthy and diabetic subjects; †significant changes versus baseline following L-arginine administration as calculated by ANOVA.

is not limited by substrate availability (27). Hence, these results likely represent the saturation point of the dose-response curve to NO production. A limitation of this finding is the slightly higher insulin plasma level in IDDM patients than in control subjects, which might influence the vascular response to L-NMMA or L-arginine. However, to study patients after an overnight fast would probably result in high glucose plasma levels, which strongly influence ocular blood flow.

The finding that L-arginine increases ocular blood flow in patients with diabetes might be of therapeutic interest in patients with diabetic retinopathy. Whereas it has been reported that in patients with early stages of retinopathy retinal blood flow is markedly (35) and choroidal blood flow slightly increased (36), patients with proliferative retinopathy have a reduced retinal and choroidal blood flow (37,38). The results obtained during L-NMMA infusion indicate that altered reactivity of ocular vessels to NO or altered local NO synthesis may be involved in the pathology of diabetic retinopathy, which has already been hypothesized, based on in vitro and animal studies (12). However, it has to be emphasized that five IDDM patients have developed mild-to-moderate diabetic retinopathy. We, therefore, cannot decide whether altered ocular reactivity to NO-synthase inhibition is the cause or effect of diabetic retinopathy. Moreover, the ~20% decrease in FPA in healthy subjects following systemic NO-synthase inhibition indicates that a sufficient NO production is required to maintain the high perfusion level in the choroid. This is in agreement with previous animal studies (9,10) and findings from our study group in healthy subjects (11).

When discussing our results, some limitations concerning the study methodology have to be mentioned. On the one hand, exhaled NO does not necessarily reflect the degree of NO production, which has already been raised above. On the other hand, fundus pulsation measurements only estimate the pulsatile blood flow component and, therefore, cannot necessarily be extrapolated to total blood flow. This limitation has been discussed in detail previously (21,39). In the present study, we found no drug-induced change in the ratio of pulsatile to non-pulsatile blood flow in the ophthalmic artery, as evidenced by Doppler ultrasound (data not shown). Hence, it can be assumed that non-pulsatile blood flow in the

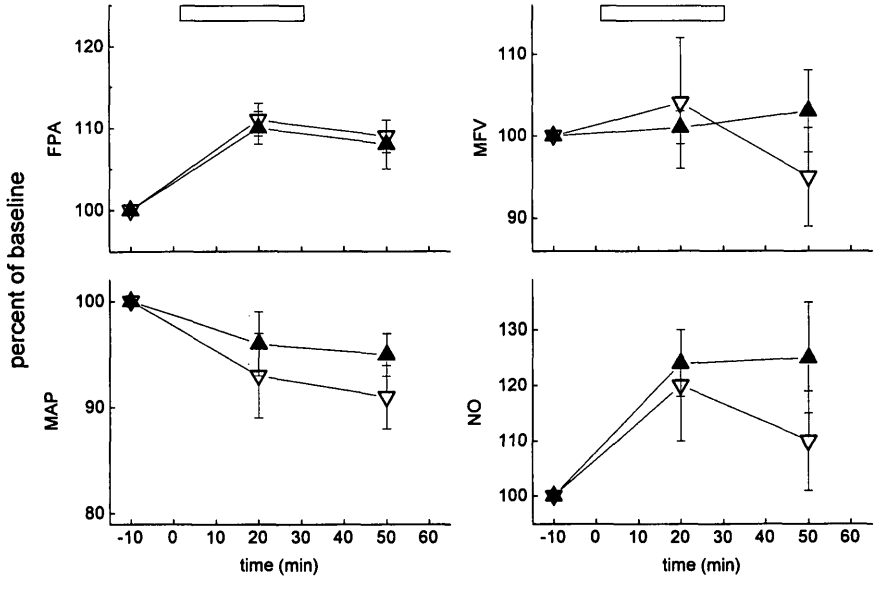

FIG. 2. Time course of the effect of systemic infusion of L-arginine (420 mg/kg over 30 min) in patients with IDDM (n = 10, ▲) and healthy control subjects (n = 10, ▽) on FPA, MAP, MFV in the ophthalmic artery, and exhaled NO. The infusion period is indicated by a box. The data are expressed as percentage of baseline. Error bars represent the standard error of the means.

choroid changed to a comparable degree as pulsatile flow. With the results obtained from Doppler ultrasound, it has to be kept in mind that the reproducibility of the measurements is limited (40). In our study, a significant difference between the two study groups was only observed 30 min after L-NMMA administration, which might at least partially be attributed to data variability.

In conclusion, we have shown that systemic and ocular hemodynamic reactivity to NO-synthase inhibition is reduced in patients with IDDM, compared with age-matched healthy subjects. This study supports the concept that either NO-synthase activity is increased or NO sensitivity is decreased in patients with IDDM and indicates that the L-arginine–NO system is likely involved in the pathophysiology of diabetic retinopathy.

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