L-Arginine–Induced Vasodilation of the Renal Vasculature Is Not Altered in Hypertensive Patients With Type 2 Diabetes

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OBJECTIVE — Diabetes, arterial hypertension, hypercholesterolemia, and aging are associated with endothelial dysfunction in various vasculatures. Endothelium-dependent vasodilation of the renal vasculature cannot be easily assessed, but infusion of L-arginine, the substrate of endothelial nitric oxide synthase, leads to an increase in renal plasma flow (RPF) in humans. We have examined the effect of L-arginine infusion on renal hemodynamics in hypertensive patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS — Twenty-three elderly patients with type 2 diabetes (age, 65 ± 6 years; HbA_{1c}, 7.8 ± 1.6%) with coexisting arterial hypertension (158 ± 19/83 ± 11 mmHg) and elevated cholesterol levels (total cholesterol, 215 ± 33 mg/dl) were examined. These patients were compared with a young and healthy reference group ($n = 20$; age, 26 ± 2 years). The effect of L-arginine infusion (100 mg/kg over 30 min) on RPF and glomerular filtration rate were measured using the constant input clearance technique with *p*-aminohippurate and inulin, respectively.

RESULTS — L-Arginine infusion similarly influenced renal hemodynamics in patients and reference subjects: RPF increased by 7 ± 11 and 7 ± 11% in diabetic and reference subjects, respectively (P = NS). Other parameters of renal hemodynamics such as glomerular filtration rate (5 ± 5 vs. 4 ± 4%) and filtration fraction (−1 ± 8 vs. −1 ± 9%) were not significantly different between diabetic and reference subjects, too.

CONCLUSIONS — L-Arginine–induced vasodilation of the renal vasculature is not different between a group of hypertensive diabetic patients and a young, healthy reference group. These data were obtained using low-dose L-arginine infusion.

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There is a large body of evidence that endothelial dysfunction is an early step in the development of atherosclerosis (1). In patients with type 2 diabetes, endothelial dysfunction has been found in the forearm and coronary vasculature and precedes both macrovascular and microvascular complications (2,3). Arterial hypertension, elevated cholesterol levels, and higher age are other independent risk factors for the development of endothelial dysfunction (4–6) and are all commonly found in patients with type 2 diabetes.

In the renal vasculature, endothelial dysfunction influences the regulatory capabilities of afferent and efferent arterioles (7). Changes of renal plasma flow (RPF), glomerular filtration rate (GFR), and even the development of proteinuria due to such changes are the consequence of endothelial dysfunction of the renal vasculature. Accordingly, endothelial dysfunction of the renal vasculature is considered an early step in the development of human diabetic nephropathy (8). One aspect of "endothelial dysfunction" is impaired endothelium-dependent vasodilation. In an animal model, impaired endothelium-dependent vasodilation of the renal artery has been found in diabetic rabbits but not in controls (9).

The assessment of endothelium-dependent vasodilation of the renal vasculature in humans is difficult. Infusions of L-arginine, the substrate of endothelial nitric oxide synthase, and subsequent measurement of changes of renal hemodynamics have been used for this purpose (10–16). However, others and we have shown that the effects of high-dose L-arginine infusion on renal hemodynamics are at least in part unspecific (10,11). Using low-dose L-arginine infusions, we did not observe a difference in L-arginine–induced vasodilation of the renal vasculature between middle-aged patients with hypercholesterolemia and a young and healthy reference group (15). The present study was conducted to gain further insight into the importance of endothelial dysfunction of the renal vasculature for the development of renal disease but also into the validity of L-arginine infusions as a tool to assess endothelium-dependent vasodilation of the renal vasculature. We have therefore compared the effects of low-dose L-arginine on renal hemodynamics between a similar young and healthy reference group and a group of elderly patients with type 2 diabetes, arterial hypertension, and elevated cholesterol levels.

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Abbreviations: GFR, glomerular filtration rate; RPF, renal plasma flow.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

RESEARCH DESIGN AND METHODS

Patients and reference subjects

Patients with type 2 diabetes (age between 60 and 75 years), arterial hypertension (systolic blood pressure above 140 mmHg and/or diastolic blood pressure above 90 mmHg according to World Health Organization criteria), and elevated serum total (above 180 mg/dl) and/or LDL cholesterol (above 130 mg/dl) were eligible for the study. The patients have been treated for type 2 diabetes and arterial hypertension in a high-risk patient program in our outpatient clinic (17) and were asked to take part in the present study. Twenty young and healthy students from the University of Erlangen-Nürnberg served as a reference group. All patients and reference subjects underwent a clinical examination. Blood pressure and laboratory parameters were determined, and 12-lead electrocardiography was performed to exclude any other severe renal, hepatic, or cardiovascular disease. In particular, patients with elevated serum creatinine (above 1.2 mg/dl) and/or overt albuminuria (above 300 mg/day) were excluded from the study. Patients were permitted to stay on their usual antidiabetic and antihypertensive medication but were asked not to take any antihypertensive drugs at the day of the clearance study. Of 17 patients, 14 received antihypertensive medication (monotherapy, double therapy, and triple therapy in 5, 3, and 6 patients, respectively; ACE inhibitors, angiotensin receptor blockers, calcium channel blockers, β−receptor blockers, diuretics, and clonidine in 9, 3, 5, 4, 7, and 1 patients, respectively) All patients and reference subjects gave their written informed consent before study inclusion. The study protocol was approved by the Clinical Investigations Ethics Committee of the University of Erlangen-Nürnberg.

Infusion protocol

Renal hemodynamic parameters were determined by constant infusion input clearance technique with inulin (Inutest, Fresenius, Linz, Austria) and sodium *p*-aminohippurate (Nephrotest, Merck, Sharp & Dohme, Hertfordshire, U.K.) for GFR and RPF, respectively. These procedures have been described previously (18). Briefly, the examination was performed in a quiet laboratory from 8:00 A.M. to 12:00 A.M. with the subject in the supine position. The participants ingested their usual breakfast and were infused 500 ml of normal saline during the examination. After bolus infusion of inulin and sodium *p*-aminohippurate over 15 min and a subsequent constant infusion over 105 min, a steady state between input and renal excretion of the tracer substances was reached (18), and the administration of experimental substances was started. Systemic hemodynamic parameters (i.e., blood pressure and heart rate) were monitored by means of an oscillometric device (Dinamap 1846 SX, Criticon, Norderstedt, Germany).

L-Arginine was administered intravenously at a dose of 100 mg/kg over 30 min. We used L-arginine hydrochloride solution 6% (manufactured at the University Hospital Pharmacy, Erlangen, Germany) and calculated the volume needed over 30 min according to the dose in each study participant (e.g., for a 70-kg participant, 117 ml per 30 min). An additional infusion of normal saline was regulated to achieve a constant volume input of 4 ml per kg of body weight per hour over the whole clearance protocol. Blood samples to determine inulin and *p*-aminohippurate concentration were drawn at 0, 120, and 150 min. During the last 5 min of each infusion step, blood pressure was monitored every minute, and the mean of these measurements is given.

Using this approach, we have previously observed an increase of RPF by 40 ± 51 and 40 ± 52 ml/min through 100 mg/kg L-arginine in middle-aged patients with hypercholesterolemia and young and healthy reference subjects, respectively (15). In five middle-aged patients (age, 57 ± 7 years; BMI, 23.8 ± 0.7 kg/m^2; blood pressure, 140 ± 18/85 ± 6 mmHg), we observed an increase in RPF by 32 ± 25 ml/min through 100 mg/kg L-arginine (C.D., M.P.S., S.O., E.H.F., R.E.S., unpublished observations). The reproducibility of the steady-state input clearance method is continuously monitored in our laboratory. Subsequent examinations of the same patients, for instance, showed a coefficient of variance of 8.6% for the determination of RPF in data from a previous study in 20 healthy young subjects (10) and a correlation coefficient of 0.95 ($P = 0.004$) between two subsequent measurements of RPF in six elderly patients with type 2 diabetes (C.D., M.P.S., S.O., E.H.F., R.E.S., unpublished observations).

Laboratory measurements

Blood samples were centrifuged immediately at 4°C and were stored at −21°C until measurement. Measurement of *p*-aminohippurate and inulin was performed after completion of the study. *p*-Aminohippurate was measured by the method of Smith et al. (19); inulin was measured indirectly with an enzymatic method after conversion to fructose. Each blood sample was measured in duplicate with a coefficient of variation of <5%.

Statistics

The hypothesis was that L-arginine at a dose of 100 mg/kg increases RPF by 7 ± 9% in the reference group (according to previous data [10,12]) and that no change in RPF will be found in the patient group. The sample size was calculated to test this hypothesis with a power of 80% at an α-level of 0.05. A Kolmogorov-Smirnov test revealed that all parameters (including renal hemodynamic parameters) with the exception of age were normally distributed across the whole study population (patients and control subjects). Analyses were therefore performed with paired and unpaired Student's *t* tests, where appropriate, using SPSS Software (release 10.0; SPSS Inc., Chicago, IL). Age was compared between the groups with the Mann-Whitney *U* test. Calculation of Pearson correlation coefficients was used to examine the effect of age on the response of renal hemodynamics to L-arginine infusion. A P value <0.05 was considered significant. All data are given as mean ± SD.

RESULTS

Baseline characteristics

Baseline characteristics of patients and reference subjects are depicted in Table 1. According to the inclusion criteria, patients had a higher systolic blood pressure ($P < 0.001$), higher cholesterol levels ($P < 0.001$), and were older than the reference subjects ($P < 0.001$). In addition, BMI was greater in patients than in reference subjects ($P < 0.001$). Baseline renal hemodynamic parameters with the exception of GFR were also different between the groups with lower RPF ($P < 0.001$), lower renal blood flow ($P < 0.01$), greater filtration fraction ($P <$

Table 1—*Baseline characteristics*

	Type 2 diabetes	Reference group
n	23	20
Age (years)	65 ± 6	26 ± 2*
BMI (kg/m^2)	31.3 ± 4.1	23.0 ± 2.4*
Duration of type 2 diabetes (years)	12.4 ± 6.4	—
Duration of arterial hypertension (years)	15 ± 13	—
Systolic blood pressure (mmHg)	158 ± 19	125 ± 7*
Diastolic blood pressure (mmHg)	83 ± 11	76 ± 8
Serum creatinine (mg/dl)	0.8 ± 0.3	0.9 ± 0.1
HbA_{1c} (%)	7.8 ± 1.6	—
Albuminuria (mg/dl)	33 ± 29	—
Serum total cholesterol (mg/dl)	215 ± 33	151 ± 31*
Serum LDL cholesterol (mg/dl)	141 ± 33	70 ± 19*
RPF (ml/min)	535 ± 119	658 ± 96*
Renal blood flow (ml/min)	934 ± 231	1,163 ± 174†
Glomerular filtration rate (ml/min)	122 ± 22	119 ± 15
Filtration fraction	0.23 ± 0.03	0.18 ± 0.03*
Renal vascular resistance (mmHg min/l)	118 ± 32	74 ± 11*

Data are means ± SE. *$P < 0.001$; †$P < 0.001$.

0.001), and greater renal vascular resistance ($P < 0.001$) in patients than in reference subjects.

Response of renal hemodynamics to L-arginine infusion

In diabetic patients, L-arginine infusion at a dose of 100 mg/kg caused an increase of RPF from 535 ± 119 to 566 ± 107 ml/min ($P < 0.05$), an increase of renal blood flow from 934 ± 231 to 986 ± 204 ml/min ($P < 0.05$), an increase of GFR from 122 ± 22 to 127 ± 21 ml/min ($P < 0.01$), and a decrease of renal vascular resistance from 118 ± 32 to 109 ± 30 mmHg min/l ($P < 0.05$). Mean arterial blood pressure was not significantly decreased by L-arginine infusion (103 ± 14 vs. 101 ± 16 mmHg, P = NS). Also, filtration fraction was not affected by L-arginine (0.23 ± 0.03 vs. 0.22 ± 0.03; P = NS).

In reference subjects, L-arginine infusion at a dose of 100 mg/kg caused an increase of RPF from 658 ± 96 to 697 ± 93 ml/min ($P < 0.05$), an increase of renal blood flow from 1,163 ± 174 to 1,236 ± 190 ml/min ($P < 0.05$), and an increase of GFR from 119 ± 15 to 124 ± 14 ml/min ($P < 0.001$). There was a trend toward a decrease of renal vascular resistance in response to L-arginine (74 ± 11 vs. 69 ± 11 mmHg min/l; P = 0.054). Mean arterial blood pressure was not significantly decreased by L-arginine infusion (85 ± 7 vs. 84 ± 7 mmHg; P = NS). Again, filtration fraction was not affected by L-arginine (0.18 ± 0.02 vs. 0.18 ± 0.03; P = NS).

Neither absolute nor relative (percentile) changes of the above parameters in response to L-arginine infusion were significantly different between patients and reference subjects (Table 2). Plotting the individual response to L-arginine in the two groups also showed a nearly complete overlap of the increase of RPF (Fig. 1). Thus, the response of renal hemodynamics appeared similar in the diabetic group and in the reference group. Also, there were no significant correlations between age and the response of renal hemodynamics to L-arginine infusion in the whole study group (data not shown).

CONCLUSIONS — Impaired endothelium-dependent vasodilation has been found in the forearm and coronary vasculature of patients with type 2 diabetes (2,3). Other risk factors for endothelial dysfunction comprise arterial hypertension and ageing, and there is evidence that these risk factors have an additive effect on endothelial dysfunction in patients with type 2 diabetes (4–6). Several lines of evidence support the hypothesis that changes in endothelial function of the renal vasculature are associated with the development of diabetic nephropathy (20,21).

To assess endothelium-dependent vasodilation of the human renal vasculature, systemic administration of L-arginine, the substrate of endothelial nitric oxide synthase, has been widely used (12). Hitherto, L-arginine has been used at a high dose of some 500 mg/kg by others and us (10,11,15,16). However, apart from the specific effect on endothelial nitric oxide synthase, high-dose L-arginine exerts several unspecific effects (10). We have therefore established low-dose L-arginine infusion (100 mg/kg) to examine endothelium-dependent vasodilation of the renal vasculature to overcome these side effects (10,12), an approach that has also been made by others (14). Of note, simply transferring the

Table 2—*Response of hemodynamic parameters to L-arginine infusion*

	Type 2 diabetes	Reference group
n	23	20
Absolute changes		
Change of mean arterial pressure (mmHg)	−2 ± 5	−1 ± 4
Change of RPF (ml/min)	+32 ± 55	+40 ± 70
Change of renal blood flow (ml/min)	+52 ± 91	+73 ± 126
Change of glomerular filtration rate (ml/min)	+5 ± 8	+5 ± 5
Change of filtration fraction	−0.00 ± 0.02	−0.00 ± 0.02
Change of renal vascular resistance (mmHg min/l)	−9 ± 16	−5 ± 10
Relative (percentile) changes		
Change of mean arterial pressure (%)	−2 ± 7	−1 ± 5
Change of RPF (%)	+7 ± 11	+7 ± 11
Change of renal blood flow (%)	+7 ± 11	+7 ± 11
Change of glomerular filtration rate (%)	+5 ± 5	+4 ± 4
Change of filtration fraction (%)	−1 ± 8	−1 ± 9
Change of renal vascular resistance (%)	−4 ± 14	−3 ± 15

Data are means ± SE. Absolute and relative (percentile) changes of hemodynamic parameters in response to infusion of L-arginine (100 mg/kg) are given. Note the absence of any significant differences between the groups.

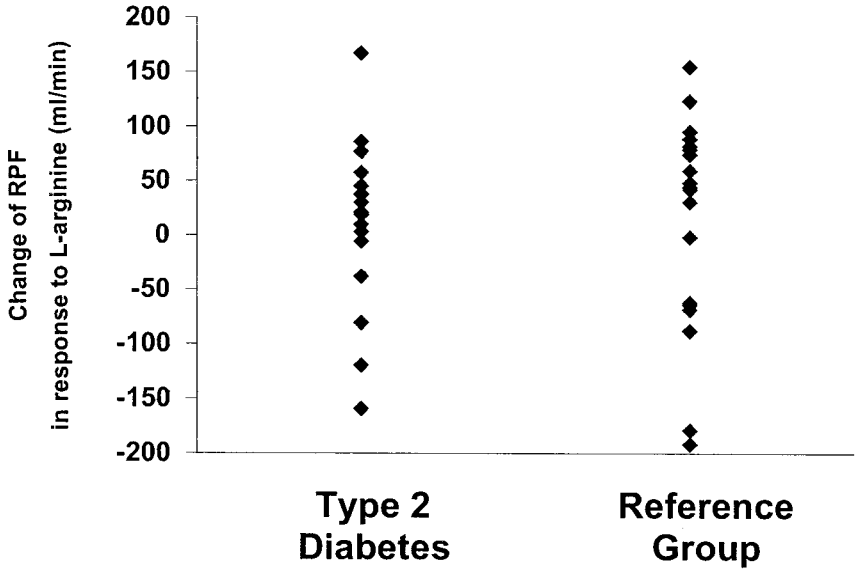

Figure 1—*Change of RPF in response to L-arginine (100 mg/kg) in individual diabetic and reference subjects. Note the absence of a significant difference between the groups.*

results from other vasculatures to the renal vasculature is not possible. For instance, we have recently demonstrated in patients with arterial hypertension that endothelium-dependent vasodilation of the forearm and renal vasculatures are not related with each other (13).

In a recent study we did not find differences in L-arginine–induced vasodilation of the renal vasculature between middle-aged hypercholesterolemic patients and young and healthy control subjects (15). Thus, we planned the present study with a similar control group but with a patient group at even higher risk for impaired endothelium-dependent vasodilation, that is, a group of elderly patients with type 2 diabetes, arterial hypertension, and elevated cholesterol levels. Following this approach, we had to accept that patients and control subjects would differ not only in their clinical inclusion criteria but also in baseline renal hemodynamic parameters that might influence the response of renal hemodynamics to L-arginine. However, the reference group was chosen in the hope to find any difference in the response of renal hemodynamics to L-arginine as compared with diabetic patients.

Our finding that L-arginine–induced vasodilation of the renal vasculature was not different between the patient and the reference group was somewhat surprising. Three potential explanations have to be discussed. First, endothelium-dependent vasodilation of the renal vasculature might not be impaired in our patients. At first glance, this explanation appears not very probable due to the high risk for endothelial dysfunction in our patients. However, there are reports of an increased nitric oxide synthesis in the renal vasculature in early diabetes, which accounts for the glomerular hyperfiltration found in this state of beginning diabetic nephropathy (7,22). Our patients did not have overt albuminuria and might in fact have normal or even increased nitric oxide synthesis of the renal vasculature and therefore a normal L-arginine–induced increase of RPF. This would clearly underscore the differences in endothelial function between various vasculatures, because in the forearm vasculature even insulin resistance in the presence of a family history of type 2 diabetes causes impaired endothelium-dependent vasodilation (23).

A second explanation for our present data is that L-arginine infusion might not be suitable as a tool to assess endothelium-dependent vasodilation of the renal vasculature in certain patients despite our own experience with low-dose L-arginine infusion. L-Arginine can be converted to L-ornithine without serving as a substrate for nitric oxide synthase when the activity of arginase is higher than that of nitric oxide synthase. Increased arginase activity has been found, for instance, in hypertensive patients (24). Such findings might provide a basis for the use of higher L-arginine concentrations to examine endothelium-dependent vasodilation of the renal vasculature. However, in our study in hypercholesterolemic patients (15), we have used high-dose L-arginine (500 mg/kg) as well and have not found a difference between patients and control subjects. In patients with disturbed tubulo-glomerular feedback, the amount of chloride delivered with the infusion of L-arginine hydrochloride might cause changes in renal hemodynamics without any involvement of the endothelium (25), which supports our approach to use lower L-arginine doses. As a third explanation for our present data, we cannot rule out an effect of preexisting antihypertensive therapy on L-arginine–induced vasodilation of the renal vasculature (26).

Our findings about L-arginine–induced vasodilation will be difficult to interpret as long as data on other aspects of endothelial dysfunction of the renal vasculature such as nitric oxide bioavailability and the contribution of oxidative stress to endothelial dysfunction are scarce in patients with type 2 diabetes. Recently, Dalla Vestra et al. (27) have found some differences between patients and control subjects in the response to infusions of a nitric oxide synthase inhibitor. We are convinced that further conclusions about endothelium-dependent vasodilation of the renal vasculature in patients with type 2 diabetes can be drawn when the effects of nitric oxide synthase inhibition and antioxidant treatment on renal hemodynamics are examined in a greater number of patients. At this moment, we can state on the basis of our data that L-arginine–induced vasodilation is not impaired in patients with type 2 diabetes and that L-arginine appears not to be a rate-limiting factor of impaired nitric oxide synthesis in diabetic patients.

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