Inhibition and Stimulation of Nitric Oxide Synthesis in the Human Forearm Arterial Bed of Patients with Insulin-dependent Diabetes

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Abstract

Patients with insulin-dependent diabetes mellitus have an increased mortality and morbidity due to vascular complications. Nitric oxide from the vascular endothelium contributes to the control of normal vascular tone, and endothelial dysfunction has been implicated in the pathogenesis of diabetic vascular disease. In this study we have examined basal and stimulated nitric oxide–mediated vasodilatation in insulin-dependent diabetics and age- and sex-matched healthy controls. Drugs were infused locally into the brachial artery and forearm blood flow measured using venous occlusion plethysmography. Noradrenaline and N^G-monomethyl-L-arginine produced similar reductions in resting forearm blood flow in healthy controls. However, in the diabetics, N^G-monomethyl-L-arginine was significantly less effective than noradrenaline. Comparing between groups, the response to N^G-monomethyl-L-arginine was also significantly less in the diabetics compared with the healthy controls. The response to sodium nitroprusside was significantly less in the diabetics compared with the healthy controls, whereas the responses to both acetylcholine and verapamil were the same in the two groups. The results provide evidence for an abnormality of basal nitric oxide–mediated dilatation in the forearm arterial bed of patients with insulin-dependent diabetes mellitus, and suggest that the vascular smooth muscle is less sensitive to nitric oxide. (*J. Clin. Invest.* 1992. 90:2548–2554.) Key words: diabetes mellitus • endothelium • forearm blood flow • human • N^G-monomethyl-L-arginine • nitric oxide • resistance vessels

Introduction

Diabetic patients have an increased morbidity and mortality due at least in part to vascular complications such as atheroma, hypertension, microangiopathy, and platelet activation (1–4). Vascular endothelium contributes to the control of vascular tone and platelet function, and endothelial dysfunction has been implicated in the pathogenesis of diabetic vascular disease (5, 6). Recently interest has focused on endothelium-derived relaxing factor (7), whose biological activity is accounted for by nitric oxide (NO)[1] (8). This mediator, which is synthesized from L-arginine (9) by NO synthase (10), is a potent vasodilator (11, 12) and inhibitor of platelet adhesion and aggregation (13, 14). Studies in animals (15–17) and human tissue in vitro (18) have suggested that endothelial NO-mediated dilatation may be impaired in diabetes. It is possible that such endothelial dysfunction contributes to the development of diabetic vascular complications (19).

NO synthesis may be continuous (basal) (20) or the result of stimulation by agonists such as acetylcholine (ACh) or bradykinin (11). In both instances NO synthesis is inhibited by certain analogues of L-arginine, including N^G-monomethyl-L-arginine (L-NMMA) (21), which acts as a competitive, stereospecific inhibitor of NO synthase (22). Systemic inhibition of NO synthesis in animals leads to vasoconstriction (23), a rise in blood pressure (24–26), and, in some vascular beds under experimental conditions, enhanced platelet adhesion and aggregation (27). In the human forearm, local infusion of L-NMMA causes a near doubling of arteriolar resistance (20), suggesting that basal NO release is an important determinant of resting blood flow. Local forearm infusion of ACh causes vasodilatation due, at least in part, to stimulation of NO synthesis (20).

In this study we have examined the L-arginine/NO pathway in a group of patients with insulin-dependent diabetes, and a group of age- and sex-matched healthy controls. We have investigated the effect on forearm blood flow of agonist-stimulated release of NO using ACh, and basal release of NO using the NO synthase blocker L-NMMA. In addition we have examined the response to sodium nitroprusside (SNP), an endothelium-independent vasodilator whose active moiety is NO (28), and to verapamil, which acts independently of the NO system.

Methods

Subjects. The study, which had the approval of the local ethical committee, was undertaken in 10 male patients with insulin-dependent diabetes mellitus and 10 age- and sex-matched normal controls. One of the diabetic patients and one of the control subjects was Afro-Caribbean, all other subjects were white. All volunteers gave their informed consent. The diabetic patients were receiving treatment with insulin only, the healthy subjects were receiving no medication. All subjects were normotensive.

Diabetic patients with recent onset of type I diabetes mellitus and no clinical or biochemical evidence of diabetic nephropathy, neuropathy, or vasculopathy were recruited from clinics at St. George's Hospital. On each study day blood pressure was measured using a semiautomatic ultrasound sphygmomanometer (Arteriosonde) immediately before forearm blood flow studies, and blood samples were taken for measurement of serum sodium, potassium, creatinine, glucose, cholesterol, liver-function tests, and glycosylated hemoglobin (HbA_{1C}; diabetics only).

Forearm blood flow studies. Studies were performed in a temperature-controlled laboratory (26–28°C) with the subjects lying supine.

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1. *Abbreviations used in this paper:* ACh, acetylcholine; AUC, area under the dose-response curve; HbA_{1C}, glycosylated hemoglobin; L-NMMA, N^G-monomethyl-L-arginine; NA, noradrenaline; NO, nitric-oxide; SNP, sodium nitroprusside.

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Forearm blood flow (ml/100 ml forearm per min) was measured simultaneously in both arms by mercury-in-rubber strain gauge plethysmography (29). Drugs or physiological saline were infused continuously into the brachial artery of the nondominant arm through a 26SWG needle introduced under local anesthetic (1% lignocaine). During the recording period the hands were excluded from the circulation by inflating a wrist cuff to a pressure of 200 mmHg. The upper arm congesting cuffs were inflated to 40 mmHg for 10 s in each 15-s cycle. At the start of each study, after inserting the needle into the brachial artery, baseline measurements of blood flow were made for 25 min to establish resting control values. During this period measurements of flow were made for 5 min in every 10 min. Throughout drug infusions blood flow was recorded continuously. Each subject was studied on two occasions separated by ≥ 7 d. To take account of any differences in basal blood flows between the two groups an internal control was used in each study, noradrenaline (NA) when studying the effects of L-NMMA on basal NO production, and verapamil when studying ACh-stimulated NO production and the sensitivity of vascular smooth muscle to exogenous NO.

Study 1. Effect of L-NMMA on basal blood flow. After resting control values of forearm blood flow had been measured, subjects received local intraarterial infusions of three doses of NA (60, 120, and 240 pmol/min each dose for 5 min) to produce a cumulative dose–response curve. 15 min later, when blood flow had returned to control values, subjects received three doses of L-NMMA (1, 2, and 4 μmol/min each dose for 5 min). 10 min after completing the infusion of L-NMMA, glyceryl trinitrate (5 nmol/min for 5 min) was infused to return blood flow to baseline. The blood flow responses to L-NMMA and NA were compared for each individual.

Study 2. Effect of stimulated NO release on forearm blood flow. After resting control values of forearm blood flow had been determined, the responses to three vasodilator drugs were measured: ACh (25, 50, and 100 nmol/min), which stimulates the production of NO by the endothelium (11), SNP (4.2, 12.6, and 37.8 nmol/min), an agent whose active moiety is NO (28), and verapamil (10, 20, and 40 nmol/min), which was used as an internal control. Each dose was given for 3 min to produce a cumulative dose–response curve, and 15 min was allowed between dose–response curves during which time blood flow returned to control values. The order of the ACh and SNP infusions was varied between subjects. Verapamil was always given last because it has a relatively long duration of action. The responses to SNP and ACh were compared with the response to verapamil for each subject.

Statistics and calculations. Forearm blood flow was expressed as ml/100 ml forearm per min according to the method of Whitney (29). The ratio of blood flow in the infused arm compared with that in the control arm was calculated for each measurement period. The ratio of forearm blood flow (infused:control arm) measured in response to drugs was expressed as a percentage of the ratio (infused:control arm) measured during the control period (30).

This method of presentation controls for the effects on forearm blood flow of external systemic factors, such as the level of arousal of the subject, which will affect blood flow in the two arms similarly, and controls for the continual small adjustments affecting the circulation of both arms that occur even at rest (30). Presentation of the results in this way avoids extraneous "noise," and ensures that only the direct effects on forearm blood flow of the locally administered drugs are taken into account (30).

The overall response to each drug was measured as the area under the dose–response curve (AUC) (31). For each individual, results were expressed in relation to an internal control. This method enables within-group comparisons to be made so that differences in vascular behavior between groups can be detected even if baseline vascular characteristics, such as resting blood flow, differ between the groups (32). Results were expressed as mean±SEM and compared using Student's *t* test for paired or unpaired observations as appropriate, where $P < 0.05$ was considered statistically significant.

Drugs. Drugs were dissolved in physiological saline (0.9% NaCl wt/vol) and passed through a FlowPore D26 bacterial filter immediately before use. The following drugs were used: acetylcholine chloride (Sigma Chemical Co., St. Louis, MO), ascorbic acid (Evans Medical Ltd., Horsham, UK), glyceryl trinitrate (American Hospital Supplies, Stevenage, Herts, UK), L-NMMA (Wellcome Research Laboratories, Beckenham, Kent, UK), NA (Levophed; Winthrop Laboratories, Guilford, Surrey, UK), SNP (David Bull Laboratories, Warwick, UK), and verapamil (Abbott Laboratories, Queensborough, Kent). Ascorbic acid was added to NA solutions to prevent autooxidation.

Results

Patient characteristics. Baseline characteristics, except glucose levels, were similar in both groups (Table I). The mean time since diagnosis of insulin-dependent diabetes in the patients was 3.25±0.99 yr, and the mean daily insulin requirement of the patients was 31±4 U. Basal blood flow in the diabetic patients was 4.35±0.19 ml/100 ml forearm per min and in the control subjects was 3.88±0.12 ml/100 ml forearm per min ($P < 0.05$).

Study 1. Effect of L-NMMA on basal forearm blood flow. L-NMMA and NA both produced a dose-dependent reduction in the forearm blood flow ratio (infused arm:control arm) in all subjects when compared with the blood flow ratio (infused arm:control arm) measured in the control period ($P < 0.001$; Fig. 1). The absolute changes in the forearm blood flow ratio in response to NA and L-NMMA are shown in Table II. In the normal subjects the responses to L-NMMA and NA were similar (P = NS comparing AUC; Fig. 2) so that L-NMMA (1, 2, and 4 μmol/min) reduced the blood flow ratio by 23±4, 38±4, and 48±4% compared with the control ratio, and NA (60, 120, and 240 pmol/min) reduced the blood flow ratio by 28±3, 40±5, and 49±5% compared with the control ratio (Fig. 1). In contrast, in the diabetic patients, the response to L-NMMA was significantly less than that to NA (Fig. 2; $P = 0.01$ comparing AUC), so that the three doses of L-NMMA reduced the forearm blood flow ratio by 16±2, 28±3, and 36±4% compared with the control ratio, and the three doses of NA reduced the forearm blood flow ratio by 33±5, 39±5, and 47±6% com-

Table I. Characteristics of Patient and Control Subjects

	Patients	Controls
Sex (n = 10)	Male	Male
Age (yr)	26.2±1.5	24.9±1.6
Age range (yr)	19–35	19–34
Median age (yr)	25	23
Weight (kg)	76.4±2.7	77.1±3.1
Weight range (kg)	60.9–90.5	65.9–101.8
Systolic blood pressure (mmHg)	124±3	119±3
Diastolic blood pressure (mmHg)	67±2	68±2
Mean blood pressure (mmHg)	86±2	85±2
Glucose (mmol/liter)	9.0±0.8	5.3±0.3
HbA_{1C} (%)	6.7±0.5	
HbA_{1C} range (%)	3.9–9.9	
Cholesterol (mmol/liter)	3.9±0.8	4.9±0.2
Creatinine (μmol/liter)	81±3	91±2
Alanine transaminase (IU/liter)	21±3	23±3
Gamma glutaryl transferase (IU/liter)	16±2	14±2

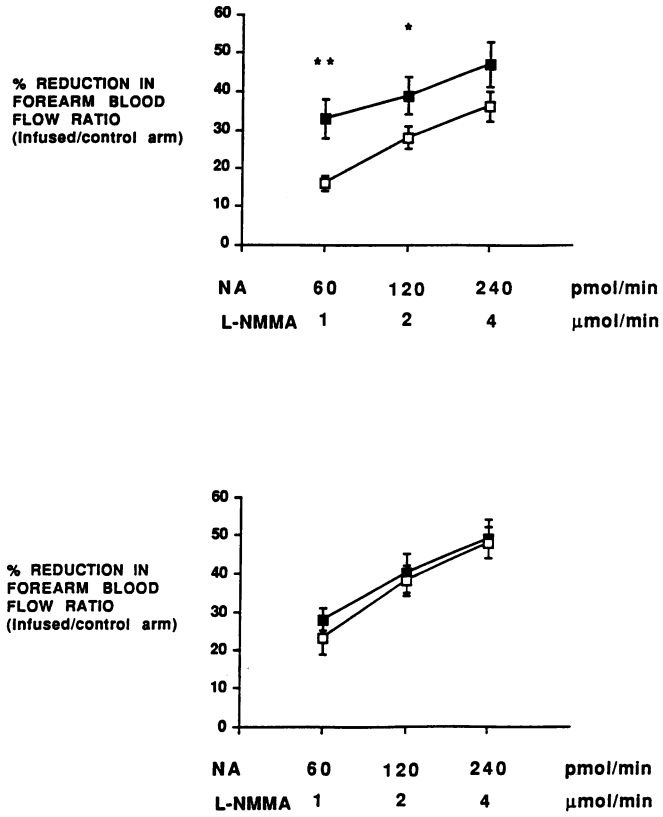

Figure 1. The percent reduction in forearm blood flow ratio (infused arm:control arm) in response to increasing doses of NA (*filled squares*) and L-NMMA (*open squares*) in diabetic patients (*top; n* = 10) and normal control subjects (*bottom; n* = 10). The response to each drug has been compared at each dose pair within the groups (**$P < 0.001$, *$P < 0.02$).

pared with the control ratio (Fig. 1). If comparisons are made between groups, there was no difference between the response to NA in the two groups (Fig. 2; P = NS comparing AUC), whereas the response to L-NMMA was significantly less in the diabetic group (Fig. 2; $P < 0.05$ comparing AUC).

In the diabetic patients there was no correlation between the response to either L-NMMA or NA and their known duration of diabetes, insulin requirements, level of glycosylated hemoglobin, or glucose. In all subjects glyceryl trinitrate returned blood flow to control values at the end of the study.

Study 2. Effect on forearm blood flow of agonist-stimulated NO release. ACh (25–100 nmol/min), SNP (4.2–37.8 nmol/min), and verapamil (10–40 nmol/min) produced dose-dependent increases in the forearm blood flow ratio (infused arm/control arm) compared with the blood flow ratio measured in the control period ($P < 0.01$). The absolute changes in the forearm blood flow ratio in response to ACh, SNP, and verapamil are shown in Table III. In the normal subjects, at the doses chosen, SNP caused a greater vasodilatation than ACh ($P < 0.05$ comparing AUC; Fig. 3), which in turn produced a greater vasodilatation than verapamil ($P < 0.02$ comparing AUC; Fig. 3). SNP increased the blood flow ratio to 275±33, 376±44, and 559±89% of the control blood flow ratio; ACh increased the blood flow ratio to 266±43, 264±36, and 314±54% of the control ratio; verapamil increased the ratio of blood flow to 134±5, 175±12, and 213±16% of the control

Table II. Absolute Changes in Forearm Blood Flow Ratio in Response to Noradrenaline and L-NMMA

	Ratio forearm blood flow*	
	Patients	Controls
Baseline	1.22±0.12	1.12±0.09
Noradrenaline, 60 pmol/min	0.83±0.12	0 81±0.08
Noradrenaline, 120 pmol/min	0.76±0.12	0.67±0.08
Noradrenaline, 240 pmol/min	0.67±0.13	0.57±0.07
Baseline	1.11±0.12	1.06±0.10
L-NMMA, 1 μmol/min	0.94 ± 0.10	0.82 ± 0.10
L-NMMA, 2 μmol/min	0.79 ± 0.08	0.67 ± 0.10
L-NMMA, 4 μmol/min	0.71 ± 0.09	0.57 ± 0.09
Within group comparison:		
Patients		
AUC noradrenaline versus AUC L-NMMA		$P < 0.05$
Controls		
AUC noradrenaline versus AUC L-NMMA		P = NS
Between group comparison:		
Noradrenaline AUC		
Patients versus controls		P = NS
L-NMMA AUC		
Patients versus controls		$P < 0.05$

* Infused/control arm; mean±SEM.

ratio. In the diabetic patients, using the same doses of drugs, ACh and SNP caused the same degree of vasodilatation (P = NS comparing AUC; Fig. 3), which was greater than that produced by verapamil ($P < 0.02$ comparing AUC; Fig. 3). SNP increased the blood flow ratio to 196±16, 256±31, and 387±59% of the control ratio; ACh increased the blood flow ratio to 210±28, 263±40, and 346±52% of the control ratio; verapamil increased the blood flow ratio to 116±4, 146±8, and 182±11% of the control ratio. Comparing the dose–response curves for each drug between diabetics and normal controls, SNP was significantly less effective in the diabetic patients (Fig. 4), whereas there was no difference detected for the responses to either ACh or verapamil. In all subjects blood flow in the control arm remained constant throughout the studies.

Discussion

The results of this study indicate that, in comparison to age- and sex-matched healthy controls, patients with insulin-dependent diabetes mellitus have a reduced forearm blood flow response to locally infused L-NMMA (an inhibitor of endogenous NO synthesis) (22) and SNP (an exogenous donor of NO) (28). Together these results suggest a diminished response to NO in the resistance vessels of the diabetic forearm.

We have examined basal NO-mediated vasodilatation and dilatation due to agonist-stimulated NO release, and in both instances have used NO-independent internal controls against which to compare the forearm response with the agents that modify the NO pathway. This is important since we found, like others before us (33, 34), that basal forearm blood flow in the diabetic patients was higher than in the healthy controls. The

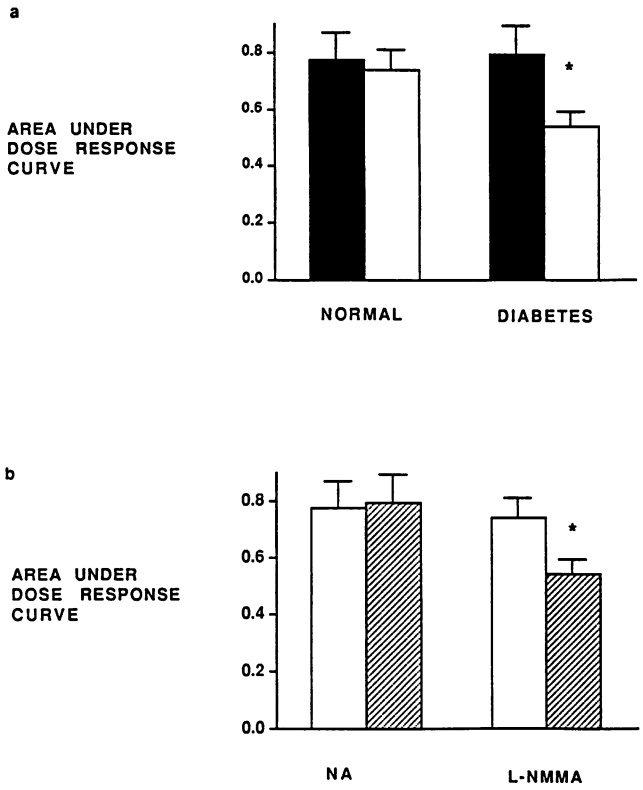

Figure 2. a) The area under the dose response curve is shown for NA (*filled columns*) and L-NMMA (*open columns*) in diabetics ($n = 10$) and normal control subjects ($n = 10$). L-NMMA was significantly less effective than NA in the diabetic patients ($*P = 0.01$) whereas the response to the two drugs did not differ in the normal subjects. *b*) The area under the NA and L-NMMA dose–response curves is shown in the normal controls (*open columns; n* = 10) and the diabetics (*shaded columns; n* = 10). The response to L-NMMA was significantly less in the diabetic patients compared with the normal controls ($*P < 0.05$) whereas the response to NA was the same in both groups.

Table III. Absolute Changes in the Forearm Blood Flow Ratio in Response to ACh, SNP, and Verapamil

	Ratio forearm blood flow*	
	Patients	Controls
Baseline	1.42±0.09	1.38±0.13
ACh, 25 nmol/min	2.85±0.30	3.52±0.52
ACh, 50 nmol/min	3.64±0.56	3.48±0.45
ACh, 100 nmol/min	4.92±0.85	4.15±0.69
Baseline	1.44±0.11	1.22±0.13
SNP, 4.2 nmol/min	2.77±0.30	3.25±0.40
SNP, 12.6 nmol/min	3.57±0.47	4.48±0.58
SNP, 37.8 nmol/min	5.42±0.90	6.51±1.02
Baseline	1.62±0.12	1.45±0.13
Verapamil, 10 nmol/min	1.85±0.12	1.92±0.14
Verapamil, 20 nmol/min	2.33±0.17	2.49±0.24
Verapamil, 40 nmol/min	2.93±0.28	3.03±0.27
Within group comparison:		
Patients		
AUC ACh versus AUC SNP		P = NS
AUC ACh versus AUC verapamil		$P < 0.02$
AUC SNP versus AUC verapamil		$P < 0.01$
Controls		
AUC ACh versus AUC SNP		$P < 0.05$
AUC ACh versus AUC verapamil		$P < 0.01$
AUC SNP versus AUC verapamil		$P < 0.001$
Between group comparison:		
ACh AUC		
Patients versus controls		P = NS
SNP AUC		
Patients versus controls		$P < 0.05$
Verapamil AUC		
Patients versus controls		P = NS

* Infused:control arm; mean±SEM.

use of an internal control in each study compensates for differences in basal conditions. Indeed, the forearm blood flow technique is at its most powerful when used to compare vascular responses and relative potencies of different drugs given sequentially within the same experiment (35). In addition, although over the course of an experiment forearm blood flow does not change significantly, even at rest small general alterations in peripheral vascular tone occur as a result of the subject's level of arousal. Such systemic factors will affect blood flow in both arms equally in percentage terms (30), but failure to control for these factors may mislead. These small general alterations in vascular tone that occur within a study can be taken into account by expressing the results in the manner of Greenfield and Patterson (30). The ratio of blood flow in the two arms (infused arm:control arm) is calculated for each measurement period, and the blood flow ratio measured in response to drugs is expressed as a percentage of the blood flow ratio measured in the control period.

The response to L-NMMA was reduced in the diabetic patients irrespective of the method of analysis; using a within-group comparison there is a relative diminution of the L-NMMA response compared with the NA response in the diabetic patients, whereas using a between-group comparison there is a reduction in the response to L-NMMA in the diabetics compared with the healthy controls. These differences are also apparent when the absolute changes in the blood flow ratio are used for comparison rather than the percentage changes.

A reduced response to L-NMMA may be interpreted in one of two ways. It might be that there is less NO synthesis occurring in the diabetic patients; consequently L-NMMA produces less vasoconstriction because NO-mediated vasodilatation is making less of a contribution to overall forearm vascular tone in these patients. Alternatively, the diabetic patients may have increased NO synthesis, and thus more L-NMMA is required to overcome the dilatation. Until accurate biochemical measurement of active NO within the vasculature becomes available it is difficult to resolve this question conclusively. However, in vitro and in vivo animal studies have been interpreted as suggesting that a reduced response to L-NMMA or other NO synthase inhibitors reflects a diminution in NO synthase activity, or effect of NO (12, 36–38). Furthermore, in studies of endotoxic shock in animals, when the level of NO synthesis is known to be increased and contributes greatly to the decreased vascular tone (39), L-NMMA produces a greater response than

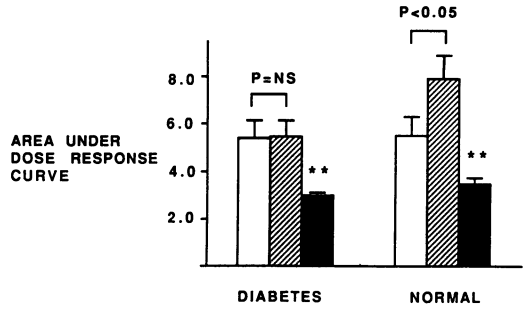

Figure 3. The area under the dose–response curve is shown for ACh (*open columns*), SNP (*shaded columns*), and verapamil (*closed columns*) in diabetic patients ($n = 10$) and control subjects ($n = 10$). In the diabetic patients the response to ACh and SNP did not differ from one another, whereas the response to both ACh and SNP was significantly greater than the response to verapamil (**$P < 0.02$). In the normal control subjects the response to SNP was significantly greater than that to ACh ($P < 0.05$) and the response to both ACh and SNP was significantly greater than that to verapamil (**$P < 0.02$).

that seen in normal animals (40). The highest dose of L-NMMA (4 μmol/min) used in the present study is at the top of the dose–response curve (unpublished observations) and thus it is more likely that a reduction in L-NMMA–induced vasoconstriction represents a diminution in the contribution made to overall forearm vascular tone by NO. This is analogous with the situation concerning other endogenous mediators such as angiotensin II, in which inhibitors of angiotensin-converting enzyme are most effective when angiotensin II levels are raised and contributing most to vascular tone (41).

The reduced response we have seen to SNP in the diabetic patients suggests that there is an abnormality in vascular smooth muscle sensitivity to NO in these patients. This contrasts with the results of a recent study in humans in vivo in which the response to SNP did not differ between normals and insulin-dependent diabetics, although such a difference was not excluded (34). However, in this earlier study, the response to SNP did diminish with increasing Na^+/Li^+ countertransport, which is a marker for the development of vascular complications in diabetic patients, and the authors conclude that the higher values of Na^+/Li^+ countertransport activity could

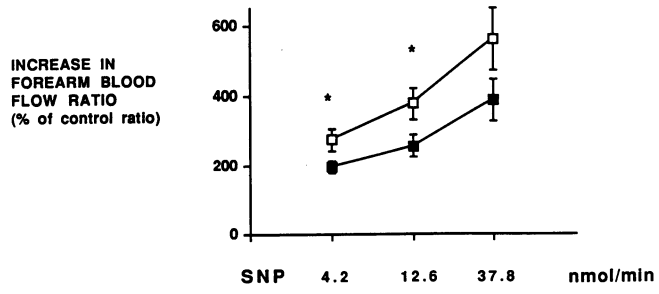

Figure 4. The increase in forearm blood flow ratio (infused arm:control arm) is shown (*% of control ratio*) in response to increasing doses of SNP in diabetic patients (*filled squares; n = 10*) and normal control subjects (*open squares; n = 10*). The response to SNP was significantly less in the diabetic patients compared with the control subjects (*$P < 0.05$). There was no difference in the dose–response curves for ACh or verapamil between the diabetic patients and the healthy controls (graphs not shown).

be linked with increased susceptibility to vascular complications as a result of diminished vascular responsiveness to endogenous endothelium-derived NO (34). Na^+/Li^+ countertransport was not measured in the present study, and it is possible that the patients we studied may have been different in this respect compared with those studied by Halkin et al. (34) and that this might account for the discrepancy in response to SNP between the two studies. Similar discrepancies exist in animal studies (15, 16, 38, 42) and the reason for these differences needs to be addressed. Animal studies have also demonstrated a progressive reduction in NO-mediated vasodilatation with increasing duration of diabetes (43). However, all the patients we studied had relatively mild hyperglycemia of comparatively short duration, and consequently it is not appropriate to compare responses in the patients we studied with those of experimental rats whose average hyperglycemia is of much greater severity.

We could detect no difference in stimulated NO release, as indicated by ACh-induced changes in forearm blood flow, between the diabetic patients and the control subjects. This finding is similar to that of others in insulin-dependent diabetes (34), whereas non–insulin-dependent diabetics may behave differently (44). Our finding of abnormal basal, but not agonist stimulated, NO release in the diabetics supports observations from studies in vitro suggesting that the NO synthases regulating basal and stimulated NO production may be different (21, 45). Furthermore, this finding serves to introduce a degree of caution when extrapolating the results of ACh infusions, which stimulate release of NO, to explain factors that might be determined by basal release of NO, such as resting peripheral vascular tone. There are additional problems with the use of ACh; the rate of ACh breakdown by plasma pseudocholinesterase may vary between subjects and the response to ACh may not be mediated entirely by NO, indeed pretreatment of the forearm with L-NMMA does not completely inhibit the vasodilator response to ACh (20).

Loss of basal NO-mediated vasodilatation would tend to favor vasoconstriction and increased peripheral resistance, at least in the short term, and could represent a mechanism to explain the increased incidence of hypertension in diabetic patients. However, the finding that blood pressure and peripheral resistance were not elevated in these patients with diabetes, in spite of the reduced basal NO-mediated vasodilatation found in this group, suggests that NO is not the only mediator on which overall long-term control of vasodilator tone depends.

Abnormalities of the L-arginine/NO pathway have also been demonstrated in humans with hyperlipidemia (46) and essential hypertension (47–49). Many vascular complications are common to essential hypertension, hyperlipidemia, and diabetes, and endothelial dysfunction with a reduction in basal NO production or a reduced sensitivity to available NO may be a common mechanism by which such apparently diverse conditions result in similar long-term vascular complications. A diminution in basal NO production or effect would tend to tip the balance in favor of vasoconstriction and platelet adhesion and aggregation and thus favor the development of vasoocclusive disorders such as ischemic heart disease, cerebrovascular disease, and peripheral vascular disease.

The mechanism by which basal NO release or effect is impaired is not clear. From our study it appears that the abnormality may lie at the level of the vascular smooth muscle in that we

observed a diminished response to SNP in the diabetic patients. Possible mechanisms for such a defect have been described in animal studies; glycosylation products that occur in diabetes may impair the NO pathway at a subendothelial level by quenching NO (43). Once advanced glycosylation has occurred it is no longer possible to restore NO-mediated vasodilatation (43). We did not find a relationship between the level of HbA_{1C} and the response to L-NMMA in our study, however, all the patients had levels of HbA_{1C} close to or within the normal range. Alternatively, altered superoxide generation in diabetes (50) may speed the destruction of NO (51) and contribute to the changes seen.

We have demonstrated an abnormality in basal NO-mediated vasodilatation in patients with insulin-dependent diabetes of relatively short duration who have no evidence of vascular complications. The abnormality appears to lie at the level of the smooth muscle, which has a reduced sensitivity to NO. If other cells that respond to NO are also less sensitive to this mediator this could contribute to increased platelet aggregation (14) and adherence to the endothelium (13) and thus to an environment conducive to the development of vascular complications. The precise contribution made by NO in disease states such as diabetes awaits the development of accurate methods for measuring the concentration of active NO released by endothelial cells in vivo.

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