Endothelium-Dependent Vascular Relaxation in Patients With Type I Diabetes

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The endothelium plays an important role in the regulation of vascular tone. Although animal data show evidence for an impaired endothelium-dependent vasodilation in diabetes, human in vivo data are scarce. We investigated 11 type I diabetic patients and 11 matched healthy control subjects. The diabetic patients were selected on their relatively poor metabolic regulation (HbA_{1c} > 8.5%), but none showed signs of microvascular complications. In all subjects, we recorded the forearm vasodilator responses to three different stimuli: *1)* 5 min of forearm ischemia to obtain a maximal vasodilator response; *2)* infusion of MCh into the brachial artery (dosages: 0.03–0.3–1.0 $\mu g \cdot min^{-1} \cdot 100\ ml^{-1}$ forearm volume) to evaluate endothelium-dependent vasodilation; and *3)* intra-arterial infusion of SNP (dosages: 0.06–0.2–0.6 $\mu g \cdot min^{-1} \cdot 100\ ml^{-1}$) to evaluate endothelium-independent vasodilation. The diabetic patients had their usual subcutaneous insulin dose and breakfast 90 min before the start of the test. Baseline levels of BP and FBF were similar in both groups. The PORH response was similar in both groups, with a percentage fall in FVR of 92 ± 1% in diabetic patients and 94 ± 1% in control subjects. In the control subjects, MCh infusions exerted a dose-dependent vasodilator response with a maximal fall in the FVR of 90 ± 2%. The highest dose of SNP induced a fall in FVR of 81 ± 6% in this group. In diabetic patients, the percentage decrements in FVR during the several dosages of MCh and SNP were similar when compared with the control group. We conclude that chronic hyperglycemia, as occurred in our patients with uncomplicated diabetes mellitus, does not impair endothelium-dependent vasodilation in vivo. *Diabetes* 42:148–53, 1993

Type I diabetes is an important and independent risk factor for the development of coronary artery disease and peripheral obstructive vascular disease. Moreover, diabetes is associated with microvascular complications in the retina, kidney, and peripheral nervous system (1). In both macro- and microvascular lesions, structural and functional abnormalities have been found in the endothelial layer of the vessels (2). In the past few years, the important role of the endothelium in the regulation of vascular tone has been established, especially by the discovery of endothelium-derived relaxing and constricting factors in several animal models (3,4). More recently, endothelium-dependent vasodilation induced by infusion of ACh also has been demonstrated in vivo in human resistance vessels and has been shown to be mediated by the production of NO (5).

Several investigators have suggested that an impaired endothelium-dependent vasodilation may contribute to or even cause the process of atherosclerosis. Within this context, it is highly interesting that an impaired endothelium-dependent vascular relaxation has been found in several experimental models for diabetes (6–8), although some investigators did not observe this endothelial dysfunction in the STZ-induced diabetic rat (9). In vitro observations in rabbits have shown that exposure to hyperglycemia results in a dysfunction of receptor-mediated endothelium-dependent relaxation of aortic rings (10). The latter observations suggest the endothelial dysfunction in diabetes may be caused at least partly by

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Type I diabetes, insulin-dependent diabetes mellitus; MCh, methacholine; SNP, sodium nitroprusside; FBF, forearm blood flow; FVR, forearm vascular resistance; PORH, postocclusive reactive hyperemia; ACh, acetylcholine; NO, nitric oxide; STZ, streptozocin; EDRF, endothelium-dependent relaxing factor; BMI, body mass index; FAV, forearm volume; cGMP, cyclic guanosine monophosphate; MAP, mean arterial pressure; BP, blood pressure; sBP, systolic blood pressure; dBP, diastolic blood pressure; AU, arbitrary unit; ANOVA, analysis of variance; NS, no significance; ANG II, angiotensin II; NE, norepinephrine.

chronic hyperglycemia. Of course, results from animal models for diabetes cannot be simply extrapolated to the human in vivo situation, especially because some investigators have observed discrepancies even in the different animal models for diabetes, showing an impaired endothelium-dependent relaxation in the diabetic BB rat without any evidence for such a defect in the STZ-induced diabetic rat (9). This discrepancy stresses the need for human in vivo studies on this item.

We hypothesize that patients with chronic hyperglycemia as a result of type I diabetes show evidence of an impaired endothelium-dependent vasodilation in vivo. To address this hypothesis, we used the isolated forearm model to measure FBF responses to intra-arterial infusion of the ACh analog, MCh, a known muscarinic receptor-mediated stimulus of EDRF release. These responses were compared with the effects of SNP, an endothelium-independent nitrovasodilator, and with the hyperemic response to forearm ischemia in both patients with type I diabetes and healthy control subjects.

RESEARCH DESIGN AND METHODS

Study population. After approval of the protocol by the local ethics committee, 11 male patients with type I diabetes and 11 healthy control subjects gave their written informed consent for participation in the study. The criteria for the diabetic patients were: duration of diabetes of >2 yr, normal serum cholesterol concentration (<6.5 mM), normal BP (<160/90 mmHg), nonsmoking, no evidence for microvascular complications such as diabetic retinopathy, neuropathy, or micro- or macroalbuminuria (nephropathy), and no clinical evidence of macrovascular disease. The patients also were selected on the basis of having a rather poor metabolic regulation with a GHb level of HbA_{1c} >8.5% to be sure of a state of chronic hyperglycemia. The control group consisted of 11 male, nonsmoking, healthy volunteers matched for age, sex, and BMI.

Study protocols. All subjects participated in one test from 0800 to 2400. The diabetic patients had their regular breakfast and prescribed morning dose of subcutaneous insulin 90 min before the start of the test, aiming representative plasma glucose concentrations throughout the test period for each individual patient. At regular intervals the plasma glucose concentrations were monitored to ensure that no unexpected hypoglycemia occurred. When the plasma glucose concentration fell below 5.0 mM, an infusion with glucose 5% was started into a right antecubital vein to prevent a further decline. The control subjects ate a light breakfast 1 h before the start of the procedure. All participants were instructed to abstain from alcohol and caffeine for at least 24 h before the experiments.

The experiments were performed in a temperature-controlled room, after an equilibration period of at least 45 min with subjects in the supine position. The left brachial artery was cannulated with a 20-gauge Angiocath (Deseret Medical, Becton Dickinson, Sandy, UT) for intra-arterial drug infusion with an automatic syringe infusion pump, (type STC-521, Terumo, Tokyo, Japan), as well as for BP and heart rate monitoring (type 78353B, Hewlett-Packard GmbH, Böblingen, West Germany). On the contralateral side, an antecubital vein was cannulated for eventual intravenous infusion of glucose 5% in the group of diabetic patients.

Throughout the test, FBF was measured at both sides by venous occlusion mercury-in-silastic strain gauge plethysmography (Hokanson EC4, D.E. Hokanson, Washington). During all recordings of FBF, the hand circulation was occluded by a wrist cuff inflated 100 mmHg above the sBP to be sure that measurements referred only to the forearm skeletal muscle vascular bed (11). The subjects remained supine throughout the study period. The dosages of all drugs were calculated per 100 ml of FAV, and, therefore, FAV of all participants was measured by water displacement.

Using bilateral plethysmographic recordings, we determined the forearm vasodilator responses to three different stimuli. Forearm ischemia was achieved by inflating a cuff around the upper arm up to 100 mmHg above the sBP for 5 min, and, subsequently, FBF recordings registered the PORH response. This PORH response has been reported to be a measure of the maximal vasodilator response (12).

We obtained new baseline FBF recordings 15 min later, and, subsequently, measured the forearm vasodilator response to intra-arterial infusion of MCh, an ACh analog. Three increasing dosages of MCh were given (0.03–0.3–1.0 $\mu g \cdot min^{-1} \cdot 100\ ml^{-1}$ FAV, 5 min per dose). By excitation of muscarinic receptors on the endothelial membrane, MCh induces an endothelium-dependent vasodilation by stimulating the generation of EDRF (13,14).

We again made baseline FBF recordings 45 min after ending the highest MCh infusion rate. Then the forearm vasodilator response to graded intra-arterial infusion of SNP (0.06–0.2–0.6 $\mu g \cdot min^{-1} \cdot 100\ ml^{-1}$ FAV, 5 min per dose) was registered. SNP was used as a control vasodilator because this drug is known to induce vasodilation not mediated by the endothelium, but directly on the smooth muscle cell using the same second messenger (cGMP) as MCh. During all baseline recordings, a placebo was infused into the brachial artery with an infusion rate similar to that of MCh and SNP to ensure an identical volume load on the forearm circulation during FBF recordings.

Drugs. SNP was purchased from Hoffmann-La Roche (Mijdrecht, The Netherlands) and dissolved in glucose 5% solution just before administration. MCh was dissolved in NaCl 0.9%. For the placebo infusion, NaCl 0.9% was used in the PORH tests and MCh infusions, whereas glucose 5% was used as a placebo just before the SNP infusions. The two drugs and the placebo infusions were administered at a rate of 50 $\mu l \cdot min^{-1} \cdot 100\ ml^{-1}$ FAV.

Data report and statistics. FBF measurements were averaged for each minute. The FVR was calculated as the quotient of the MAP and the FBF and expressed in AUs. For evaluation of the vasodilator response to forearm ischemia, we took the first and maximal FBF recording just after deflation of the cuff, and we calculated

TABLE 1
Demographic data of the healthy control subjects and diabetic patients

	Control subjects	Diabetic patients
Sex (men/women)	11/0	11/0
Age (yr)	25.8 ± 6.0 (19–39)	27.2 ± 4.7 (19–33)
Weight (kg)	73.0 ± 9.2 (55.0–85.5)	78.3 ± 6.8 (69.2–87.5)
Height (m)	1.80 ± 0.05 (1.73–1.87)	1.80 ± 0.06 (1.69–1.90)
BMI (kg/m^2)	22.6 ± 2.5 (18.2–25.8)	24.1 ± 1.4 (20.9–26.1)
FAV (ml)	1063 ± 249 (800–1600)	1036 ± 119 (950–1300)
sBP (mmHg)*	113.6 ± 15.2 (91–147)	118.9 ± 9.0 (102–133)
dBP (mmHg)*	68.4 ± 10.7 (49–84)	66.8 ± 8.8 (53–89)
Heart rate (beats/min)	63 ± 6 (48–76)	65 ± 9 (58–78)
Serum cholesterol (mM)	4.4 ± 0.9 (2.9–5.7)	4.6 ± 0.8 (3.0–5.7)
Serum triglycerides (mM)	1.38 ± 0.88 (0.59–3.40)	0.90 ± 0.43 (0.46–2.00)
Duration of diabetes (yr)	—	15.1 ± 8.2 (4–24)
HbA_{1c} (%)	—	9.2 ± 0.9 (8.5–10.6)

Values are means ± SD, and ranges are in parentheses.
*Baseline value measured by the intra-arterial line in supine position.

absolute and percentage changes by using the preceding mean baseline FBF and FVR.

For evaluation of the vasodilator response to MCh and SNP, we averaged FBF recordings of the last 2 min of each infusion rate to get the mean representative value. During these 2-min periods, FBF showed a steady state for all infusion rates used. Again, we calculated absolute and percentage changes by using the preceding baseline recordings.

Differences in baseline levels between the diabetic patients and the control subjects were evaluated with an unpaired Student's *t* test. We evaluated differences in the vasodilator responses to the various drugs and dosages between both groups using a two-way, repeated measures ANOVA. Differences were considered statistically significant at $P < 0.05$ (two-tailed). All results are presented as mean values ± SE, unless otherwise indicated.

RESULTS

Table 1 summarizes the characteristics of the diabetic patients and the healthy control subjects. The demographic data did not differ between groups. The mean HbA_{1c} at the time of the experiments averaged 9.2 ± 0.9% in the diabetic group, with reference values of 4.2–6.3% in our laboratory. The duration of diabetes ranged from 4–24 yr. At baseline, BP and heart rate were similar in both groups.

The baseline MAP on the test day averaged 85.8 ± 3.4 and 85.1 ± 2.0 mmHg in the diabetic and control groups, respectively. The baseline FBF at the experimental side was 1.5 ± 0.1 in the diabetic patients and 1.3 ± 0.1 $ml \cdot min^{-1} \cdot 100^{-1}$ ml FAV in the control subjects. Consequently, a slightly lower baseline FVR was observed in the diabetic group when compared with the control subjects (60.4 ± 3.9 vs. 70.5 ± 4.9 AU, $P > 0.10$).

Table 2 shows the mean values of the hemodynamic parameters before, during, and after the PORH test, and during each last min of the subsequent placebo/drug infusions. After 5 min of forearm ischemia, the mean maximal increase in FBF averaged 16.5 ± 0.9 $ml \cdot min^{-1} \cdot 100\ ml^{-1}$ FAV in the diabetic patients and was significantly lower than the corresponding increase of 20.3 ± 1.7 $ml \cdot min^{-1} \cdot 100\ ml^{-1}$ FAV in the control group ($P < 0.05$). Figure 1 presents the percentage changes from baseline for the calculated FVR during the several procedures. As shown in the left panel, the mean percentage decrease in FVR during PORH did not differ between the groups and averaged −92 ± 1 and −94 ± 1% in the diabetic patients and the control subjects, respectively (NS). Within a few min, the FBF declined rapidly, with a mean FBF of 2.3 ± 0.2 $ml \cdot min^{-1} \cdot 100\ ml^{-1}$ in the diabetic patients and 1.6 ± 0.1 $ml \cdot min^{-1} \cdot 100\ ml^{-1}$ in the control group over the 4th min after deflation of the cuff.

Figure 2 shows the course of the FBF during the various infusion rates of MCh and SNP. Intra-arterial infusion of MCh induced a dose-dependent increase in FBF, averaging 11.2 ± 1.3 $ml \cdot min^{-1} \cdot 100\ ml^{-1}$ FAV at the highest infusion rate in the diabetic patients versus 12.9 ± 1.9 $ml \cdot min^{-1} \cdot 100\ ml^{-1}$ FAV in the control subjects (NS). Figure 3 shows the corresponding data on the calculated FVR. The mean baseline levels of FVR after the PORH response and before the MCh infusion averaged 64.3 ± 5.1 AU in the control subjects versus 57.5 ± 4.2 AU in the diabetic patients (NS). As shown in the middle panel of Fig. 2, the mean MCh-induced percentage decrements in FVR were almost equal in both groups for all three dosages, measuring −88 ± 2 and −90 ± 2% during the highest dose in the diabetic and control subjects, respectively. Repeated measures ANOVA revealed no significant group differences in the responses of FBF and FVR to the three MCh infusion rates. After ending the MCh infusion, FBF returned to baseline levels within 45 min. At this point, the levels of FVR were slightly higher for the control subjects compared with the type I diabetic patients, averaging 93.6 ± 13.8 and 70.8 ± 8.0 AU ($P < 0.05$), respectively.

Subsequent intra-arterial administration of SNP induced a dose-dependent vasodilator response in both groups. The mean increase in FBF at the highest infusion rate of SNP averaged 5.7 ± 0.9 $ml \cdot min^{-1} \cdot 100\ ml^{-1}$ in the diabetic patients vs. 7.1 ± 1.1 $ml \cdot min^{-1} \cdot 100\ ml^{-1}$ FAV in the control subjects. The corresponding percent-

TABLE 2
MAP, heart rate, FBF (left side/experimental arm), and FVR (left and right side), before and after the PORH, and during each last minute of infusion of the placebos, and the three MCh (MCh-1 to MCh-3) and SNP dosages (SNP-1 to SNP-3) in both control subjects and diabetic patients

	MAP (mmHg)	Heart rate (beats/min)	FBF-left (ml · min^{-1} · 100 ml^{-1})	FVR-left (AU)	FVR-right (AU)
Control subjects					
Placebo	85.8 ± 3.4	65.2 ± 2.1	1.3 ± 0.2	73.8 ± 5.9	58.7 ± 7.5
PORH-1st min	84.9 ± 3.5	66.3 ± 1.9	13.1 ± 1.2	7.0 ± 0.6	52.8 ± 5.0
PORH-4th min	84.6 ± 3.6	66.4 ± 2.0	2.3 ± 0.2	40.9 ± 2.5	45.6 ± 2.8
Placebo	87.2 ± 3.7	62.5 ± 3.1	1.1 ± 0.1	86.5 ± 9.0	67.4 ± 8.2
MCh-1	87.1 ± 3.9	63.2 ± 2.1	3.9 ± 0.5	24.7 ± 3.0	62.1 ± 4.1
MCh-2	87.5 ± 4.2	64.4 ± 2.3	8.7 ± 1.3	14.3 ± 3.2	61.8 ± 4.9
MCh-3	87.9 ± 4.2	63.7 ± 2.8	14.2 ± 1.9	7.4 ± 1.1	59.3 ± 3.4
Placebo	93.6 ± 4.9	62.6 ± 2.3	1.2 ± 0.2	98.7 ± 17.6	76.2 ± 4.5
SNP-1	94.3 ± 5.1	65.6 ± 2.8	3.1 ± 0.3	36.1 ± 4.5	71.0 ± 6.3
SNP-2	92.8 ± 4.7	65.2 ± 2.5	4.8 ± 0.4	20.7 ± 2.5	78.9 ± 6.9
SNP-3	93.8 ± 5.1	63.6 ± 3.8	8.4 ± 1.1	15.3 ± 4.2	79.1 ± 8.6
Diabetic patients					
Placebo	84.7 ± 1.9	66.3 ± 2.1	1.4 ± 0.1	62.4 ± 4.1	68.2 ± 8.3
PORH-1st min*	83.0 ± 2.2	66.7 ± 3.1	12.8 ± 0.9	6.8 ± 0.4	67.9 ± 5.9
PORH-4th min*	84.4 ± 2.0	66.4 ± 2.7	1.6 ± 0.1	54.5 ± 2.2	67.8 ± 8.4
Placebo	85.9 ± 2.4	64.4 ± 2.4	1.2 ± 0.1	73.6 ± 6.2	71.3 ± 7.9
MCh-1	87.9 ± 2.2	64.8 ± 2.4	3.3 ± 0.3	28.5 ± 2.7	77.4 ± 7.8
MCh-2	89.1 ± 2.4	65.1 ± 2.7	7.7 ± 1.0	14.2 ± 2.2	73.9 ± 7.7
MCh-3	89.2 ± 2.7	66.2 ± 2.9	12.6 ± 1.3	8.4 ± 1.5	75.6 ± 6.4
Placebo	93.2 ± 2.5	65.0 ± 2.6	1.5 ± 0.1	70.7 ± 9.3	88.5 ± 11.1
SNP-1	94.6 ± 2.3	65.6 ± 2.6	2.5 ± 0.2	41.1 ± 4.6	75.1 ± 4.5
SNP-2	94.4 ± 2.5	66.2 ± 2.9	4.4 ± 0.4	24.3 ± 2.9	81.0 ± 6.8
SNP-3	95.6 ± 3.0	66.3 ± 2.8	7.4 ± 1.1	16.3 ± 2.6	85.2 ± 7.4

Values are means ± SE.
*For the PORH test, mean values during the 1st and 4th min are given, and so the first (maximal) FBF is not presented in this table.

age decrements in FVR were similar in both groups, amounting to −77 ± 2 versus −81 ± 6% (right panel of Fig. 2). Again, we observed no differences between groups when the responses to the three SNP dosages were analyzed by repeated measures ANOVA.

During the various procedures, no relevant changes in the contralateral FBF occurred (Fig. 4). In the diabetic patients, the highest dose of MCh and SNP induced a change in the contralateral FBF of −0.1 ± 0.1 and 0.0 ± 0.1 ml · min^{-1} · 100 ml^{-1} FAV, respectively. In the control group, the contralateral FBF showed similar changes throughout the infusions. The nonexperimental arm showed no changes in the FVR (Table 2). The systemic MAP showed an increase during the test from 85.2 ± 2.1 to 95.6 ± 3.0 mmHg in the diabetic patients, and from 85.4 ± 3.4 to 93.8 ± 5.1 mmHg in the control subjects. This rise in BP did not seem to be related to any specific drug infusion but rather showed a gradual rise throughout the test.

The mean plasma glucose level was 11.8 ± 4.4 mM at

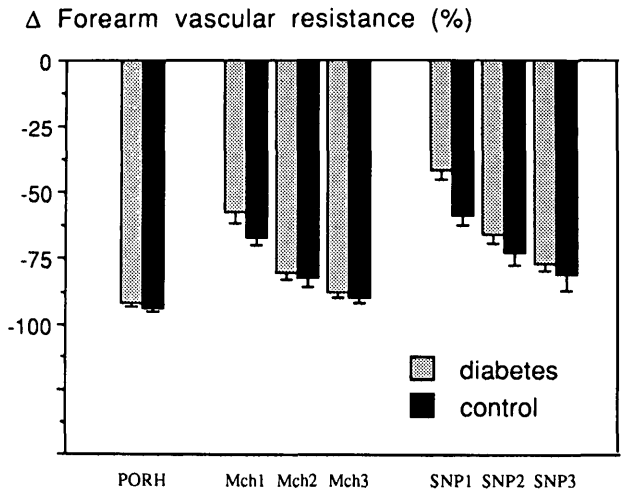

FIG. 1. The mean ± SE percentage decrements in FVR induced by forearm ischemia (PORH), three dosages of MCh (Mch1, 2, and 3), and three dosages of SNP (SNP1, 2 and 3) in control subjects and patients with type I diabetes.

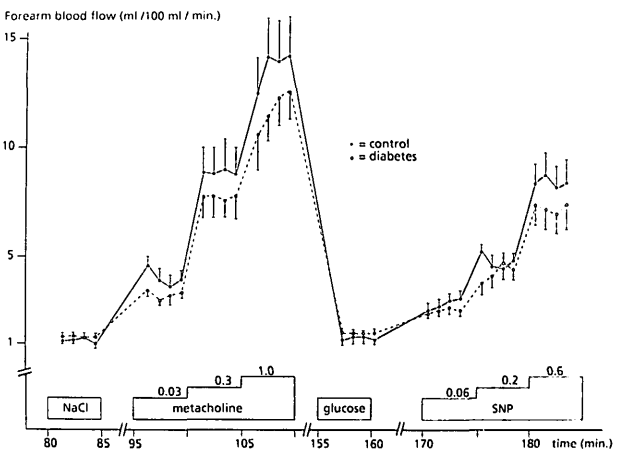

FIG. 2. The course of the mean ± SE FBF before, during, and after intra-arterial infusion of three increasing dosages of MCh and SNP in healthy control subjects (n = 11) and type I diabetes patients (n = 11).

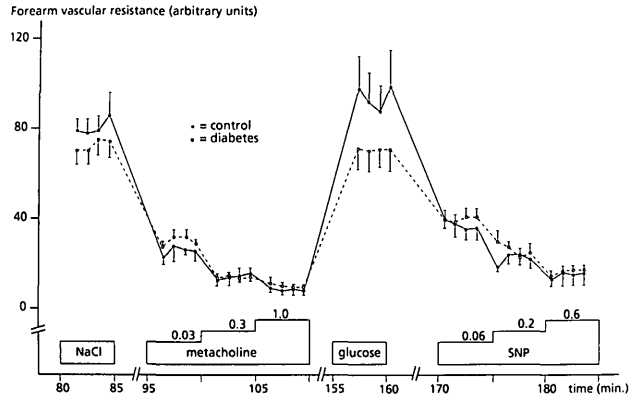

FIG. 3. The course of the mean ± SE FVR before, during, and after intra-arterial infusion of three increasing dosages of MCh and SNP in healthy control subjects (n = 11) and in diabetic patients (n = 11).

the start of the test, and throughout the experiment showed a slight decline to 9.0 ± 3.8 mM. In 2 of 11 diabetic patients, intravenous glucose infusion was necessary during the experiments to prevent a fall of the plasma glucose concentration below 5.0 mM. A subgroup of five subjects with the highst plasma glucose concentrations (15.9 ± 2.5 mM at the beginning of the experiment) showed a gradual fall to 12.3 ± 2.0 mM at the end of the procedures. When all calculations on the forearm vasodilator responses to the various stimuli were repeated for this subgroup only, we still found no differences between diabetic patients and control subjects.

DISCUSSION

Our group of diabetic patients does not show any evidence of a specific impairment of endothelium-dependent vascular relaxation in vivo. Although the absolute increments in FBF during the various stimuli seemed slightly higher in the control group than in the diabetic patients, this was significant for only the PORH response. The small and insignificant differences in the vasodilator response to the infused drugs between control subjects and diabetic patients were similar for the MCh and SNP tests, arguing against a specific defect in endothelium-dependent vascular relaxation in our diabetic patients. In fact, we were looking for differences in vascular tone, so the response of the calculated FVR is a more reliable measure of the vasodilator response than FBF. Therefore, we have based our results on the responses of FVR to the various stimuli, and especially on the percentage changes because the baseline levels of the FVR were slightly higher in the control group.

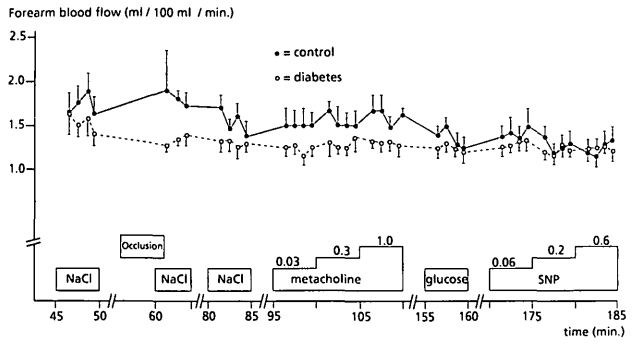

FIG. 4. Mean ± SE FBF of the nonexperimental arm before, during, and after the several test procedures.

As presented in Fig. 1, these percentage decrements in the calculated FVR were almost identical in both groups. Furthermore, no relevant changes occurred in the FBF on the nonexperimental side, making reflex adjustment as a result of the slightly increased MAP unlikely. Consequently, both the endothelium-dependent and -independent vasodilation may be interpreted as normal in our group of diabetic patients. Therefore, chronic hyperglycemia of 10–15 mM seems to be associated with neither a defect in endothelium-dependent vascular relaxation nor structural changes of the forearm vascular bed.

In the literature, cardiovascular disease states such as hypertension, hypercholesterolemia, and heart failure have been associated with an impaired endothelium-dependent vascular relaxation (12–14). Consequently, we excluded these conditions from our study. Because we also excluded microvascular complications, the main difference between the two participating groups is the state of diabetes with chronic hyperglycemia. In vitro, hyperglycemia has been shown to affect the vascular relaxation to ACh in rabbit aorta rings (10). The impaired relaxation to ACh occurs both in aorta rings from diabetic rabbits (15) and in rings from normal rabbits exposed to elevated glucose for 6 h (16).

Our observations in humans seem to disagree with these animal in vitro data. However, an important difference between the in vitro data of the aforementioned studies of Tesfamariam et al. (15,16) and our results is the level of hyperglycemia. In vitro, an impaired relaxation of rabbit aorta rings to ACh occurred only at glucose levels of ≥22 mM. In several other animal studies showing an impaired vascular relaxation to ACh, the plasma glucose levels were very high, ranging from 18 to 30 mM (6–8, 17–19). Within this context, we should stress that Tesfamariam et al. (15,16) found no differences in ACh-induced vasodilation between aorta rings incubated in a medium of 5.5 and those incubated in 11.0 mM.

In fact, the glucose levels of our patients during the several procedures averaged about 12 mM, and, as such, our data still may be in accordance with the observations of Tesfamariam et al. (15,16): Moderate hyperglycemia does not attenuate the vascular response to ACh in vitro in rabbits or in vivo in man. Even in our subgroup of diabetic patients with the highest plasma glucose concentrations, averaging ~16 mM, we found no evidence of an impaired endothelium-dependent vasodilation.

It is not known whether higher glucose concentrations of ≥20 mM result in an impaired endothelium-dependent vascular relaxation in the human in vivo situation. Long-term hyperglycemic clamp studies, or studies that use more poorly controlled diabetic patients, may elucidate this problem. Another relevant next step in the research of endothelial function in diabetes would be the expansion of our diabetic population to subjects with an early

development of microvascular disease in the eye, kidney, and/or peripheral nervous system.

Few other studies have shown human data on endothelium-dependent vasodilation in diabetes (20,21). Although Saenz de Tejada et al. (20) convincingly showed an impaired endothelium-dependent vascular relaxation in patients with diabetes, their data refer to in vitro tests of corpus cavernosum endothelium in patients with severe microvascular complications. Our data show that the findings of Saenz de Tejada et al. (20) cannot be extrapolated to in vivo data on diabetic patients without micro- or macrovascular disease.

A recent study by Halkin et al. (21) further supports this view, because these investigators did not find any difference between control subjects and diabetic patients in the vasodilator response to the ACh analog, carbachol. However, the interpretation of their data is difficult because carbachol and SNP were administered just after previous experiments with vasoconstrictor agents (NE and ANG II), which may have resulted in carry-over effects, especially because the time intervals between the four different drugs were only 5 min (21). Unfortunately, we cannot exclude eventual carry-over effects in our study because all procedures were performed in a fixed sequence: PORH, MCh infusions, SNP infusions. However, we did include equilibration periods of 15 and 45 min, respectively, after the PORH test and the MCh infusions to allow the vascular tone to return to baseline levels.

Some of our diabetic patients experienced a slight fall in plasma glucose concentrations throughout the test. Previous studies suggest that changes in plasma glucose concentrations may alter the blood flow through the microvascular bed of the skin (22). Recent investigations, however, have shown that short-term changes in plasma glucose from 5.0 to 15.0 mM for 1 h do not seem to alter the blood flow or the vascular resistance in the human forearm vascular bed (23). Furthermore, we did not observe any change in baseline blood flow in the contralateral nonexperimental arm throughout the test procedures. Consequently, we believe that our results were not influenced by the slight, short-term alterations in plasma glucose levels during our study. We realize these slight changes in plasma glucose concentrations could have been avoided by using a hyperglycemic clamp technique. With our approach, however, the results refer to a more clinically relevant condition, because the patients used their own regular insulin dose and breakfast on the morning of the test.

Chronic hyperglycemia of 10 to 15 mM, as occurs in patients with relatively poorly regulated type I diabetes, does not seem to be associated with a specific defect in endothelium-dependent vascular relaxation or with structural changes in the forearm vascular bed. Further studies are needed to find out whether such defects occur at higher levels of hyperglycemia or in subgroups of diabetic patients with evidence of microvascular disease.

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