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# A disturbed macrocirculatory supply as a determinant for a reduced sciatic nerve blood flow in diabetic rats

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**Abstract**

The aim of this study was to evaluate macrocirculatory disturbances in relation to the reduced sciatic nerve blood flow seen in diabetic rats. Therefore, both femoral blood flow, the macrocirculatory arterial blood supply to the sciatic nerve, and the microcirculatory neuronal blood flow were measured. In order to differentiate between a direct vascular or a neuronal defect as a cause for the disturbed macrocirculatory blood flow the effects of the adrenocorticotropic hormone [ACTH]-(4–9) analogue, Org 2766, a neurotrophic compound without cardiovascular effects, were investigated on the femoral flow under basal as well as adrenergic-stimulated conditions. Adrenergic responsiveness to tyramine and phenylephrine effect on femoral flow was determined. Basal sciatic nerve and femoral blood flow were reduced by 48% and 42%, respectively, after 12 weeks of diabetes, without effect on blood pressure. Treatment with Org 2766, beginning 6 weeks after the induction of diabetes, had no influence on these basal haemodynamic variables. Femoral flow in diabetic rats showed a smaller response to tyramine and phenylephrine compared to the control. Org 2766 restored this disturbed flow response to that of the control rats. In conclusion, the decrease in basal femoral flow might be responsible for the lowered sciatic nerve blood flow. Although neuronal disturbances due to diabetes had a very minor role in the reduction of basal femoral blood flow the adrenergic-stimulated flow responsiveness was seriously affected in diabetic rats.

*Keywords:* Streptozotocin-diabetic rat; ACTH-(4–9) analogue; Org 2766; Adrenergic responsiveness; Nerve blood flow; Macrocirculatory blood flow

## 1. Introduction

Evidence is accumulating that microangiopathy is important in the pathogenesis of diabetic neuropathy. Several investigators have reported alterations in microvascular blood flow and in vasoreactivity of diabetic microvessels (Fortes et al., 1983; Stevens et al., 1991). We and other groups have shown a decrease in microcirculatory nerve blood flow (Kappelle et al., 1993, 1994a; Stevens et al., 1994; Van Buren et al., 1996), and it is suggested that the vasa nervorum are affected in diabetic rats (Cameron et al., 1991). Our results indicate that disturbed neuronal control of the vasa nervorum might participate in an impairment of nerve blood flow (Kappelle et al., 1993, 1994a; Van Buren et al., 1996), namely a hyporesponsiveness to sympathoadrenergic stimuli. However, it is not known how a dysfunctional sympathetic innervation decreases sciatic nerve blood flow. Since sympathetic dysfunction would lead to a reduced $\alpha$-adrenoceptor-mediated vasoconstriction, an increase rather than a decrease in sciatic nerve blood flow would be expected. There is evidence of an association between microangiopathy (retinopathy) and macroangiopathy, e.g., impaired peripheral arterial circulation (Riccardi et al., 1988). Therefore, in an analogous way, macrocirculatory disturbances might lead to a reduced microcirculatory sciatic nerve blood flow as well. Abnormalities of cardiovascular reactivity are known to occur in diabetes mellitus. The literature on the adrenergic responsiveness of systemic blood vessels in diabetes is mostly limited to in vitro studies, e.g., on the aorta and the hindquarters (Fried-

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man, 1989; Kamata et al., 1989; Cameron and Cotter, 1992). A few in vivo studies also showed an impaired responsiveness of the cardiovascular system to sympathetic stimuli (Jackson and Carrier, 1983; Lucas, 1985; Chang and Lund, 1986; Hill and Larkins, 1989; Van der Zee et al., 1990; Kappelle et al., 1994b). To our knowledge no studies have been reported on the adrenergic reactivity of femoral blood flow, which represents the macrocirculatory supply to the sciatic nerve blood flow (Nukada et al., 1993).

It has been demonstrated that the putative neurotrophic synthetic adrenocorticotropic hormone [ACTH]-(4–9) analogue, Org 2766, is effective in peripheral nerve disorders in both rats and humans (Gispen, 1990). Van der Zee et al. (1989) and Bravenboer et al. (1993) showed that Org 2766 can protect against experimentally induced peripheral diabetic neuropathy. Recently, we reported that Org 2766 does not have a direct effect on the cardiovascular system (De Wildt et al., 1993) and is capable of improving the disturbed adrenergic responsiveness of the sciatic nerve blood flow of the streptozotocin-induced diabetic rat, and exerts its neurotrophic effects mainly at the presynaptic level (Van Buren et al., 1996).

The present study examined alterations in the macrocirculation of the femoral artery and its adrenergic responsiveness in vivo, changes which might explain the reduced sciatic nerve blood flow seen in streptozotocin-diabetic rats. In order to differentiate between a direct vascular or a neuronal defect as a cause for the disturbed macrocirculatory blood flow, the effects of Org 2766 on femoral blood flow were investigated under basal as well as adrenergic-stimulated conditions.

## 2. Materials and methods

### 2.1. Animals

All experimental protocols were approved by the Ethical Committee on the use of experimental animals of the Faculty of Medicine of Utrecht University. Male rats of an inbred Wistar strain (TNO, Zeist, Netherlands) weighing approximately 300 g were housed in Macrolon cages (2 rats per cage). The rats were maintained on a 12-h light/dark cycle (light on at 07:00 h). All rats received water and standard rodent chow ad libitum. Diabetes mellitus was induced by a single injection of streptozotocin (Serva) at a dose of 40 mg/kg in a tail vein. Diabetes was verified by measuring glucose concentrations in blood samples taken from the tail vein. An Ames Glucose Test Pack with disposable cuvettes and enzyme reagent for use with a Compur Minilab 1-photometer (Bayer Diagnostic, Munich, Germany) was used. Rats with blood glucose concentrations higher than 15 mmol/l, 48 h after the administration of streptozotocin, were considered diabetic. The non-diabetic control rats were given a food-restricted diet for 12 weeks (14 g rat chow/24 h) by which their caloric intake was adjusted to keep their body weights similar to those of the diabetic rats. During the course of the study seven animals died, two in group 1, three in group 2 and two in group 3 (see the outline of the study for groups). Data obtained from these rats were excluded from the final analysis.

### 2.2. Drugs

Org 2766, an ACTH-(4–9) analogue (H-Met-($O_2$)-Glu-His-Phe-D-Lys-Phe-OH), was a gift from Organon International (Oss, Netherlands). The peptide was dissolved in saline and administered s.c. at a dosage of 75 μg/kg every 48 h (Van Buren et al., 1996). The control rats received 0.5 ml saline. Tyramine hydrochloride, 98% (Janssen Chimica, Tilburg, Netherlands); 30, 100, 300, 500 and 700 μg/kg, an indirect sympathomimetic, and L-phenylephrine hydrochloride (Sigma, St. Louis, MO, USA); 1, 3, 10, 30, 50 and 70 μg/kg, an α-adrenoceptor agonist, were dissolved in saline and administered i.v.

### 2.3. Preparation and measurements

The rats were anaesthetized with urethane (10% dissolved in 0.9% NaCl solution) injected i.p. at a dose of 1.2 ml/100 g body weight. Urethane was used instead of barbiturates in order to ensure a minimal effect of anaesthetic on the cardiovascular system and to leave autonomic reflexes intact. The right jugular vein was cannulated with a polyethylene catheter (PE-50, Intramedic®) for intravenous injections of vasoactive agents. The left carotid artery was also cannulated with a PE-50 catheter for recording of blood pressure. The left femoral artery was dissected free and a small perivascular probe (size 1RB, Transonic Systems) was placed around the vessel and filled with acoustic gel. The flowmeter was connected to a data acquisition/processing system (see below). This method of Ultrasound Transit-time measurement of blood flow has been used for clinical and animal experimental research and has been demonstrated to be very accurate and to give reproducible results. It was also found to be a reliable technique (Honda et al., 1988; De Wildt et al., 1995) for our purpose, the measurement of blood flow in conduit vessels of the rat. Body temperature was recorded with a rectal probe and was maintained at 37°C by using a homeothermic blanket system (Harvard Apparatus). Basal haemodynamic variables were measured after a 15-min stabilization period. The change in mean arterial pressure after an i.v. bolus injection of tyramine (30–700 μg/kg) and phenylephrine (1–70 μg/kg) was recorded. The injection volume was 100 μl; injection of this volume of saline had no haemodynamic effects. An interval of 5 min was allowed between the administration of the different dosages of tyramine and phenylephrine so that blood pressure and heart rate could return to baseline. There was a 15-min

Table 1
Mean body weights and blood glucose levels

| Group | Glucose (mmol/l) | | |
|---|---|---|---|
| | 0 weeks | 6 weeks | 12 weeks |
| Diet | 5.8 ± 1.2 | 6.3 ± 1.0 | 5.8 ± 1.0 |
| DMOrg | 25.3 ± 1.5 [a] | 26.2 ± 1.1 [a] | 23.8 ± 1.8 [a] |
| DMPla | 23.7 ± 2.0 [a] | 25.2 ± 1.3 [a] | 24.9 ± 2.0 [a] |
| | Body weight (g) | | |
| | 0 weeks | 6 weeks | 12 weeks |
| Diet | 287 ± 10 | 275 ± 10 | 260 ± 12 |
| DMOrg | 300 ± 9 | 263 ± 13 | 252 ± 10 |
| DMPla | 305 ± 11 | 275 ± 10 | 248 ± 13 |

Mean blood glucose levels (Glucose) at week 0 (0 weeks, onset of diabetes mellitus), week 6 (6 weeks, start treatment) and week 12 (12 weeks, end of the experiment), and body weight at 0 wk, 6 wk and 12 wk ± S.E.M. of the non-diabetic group (Diet), the Org 2766-treated diabetic group (DMOrg) and the placebo-treated diabetic rats (DMPla). [a] Statistically significant difference from non-diabetic controls, $P < 0.001$.

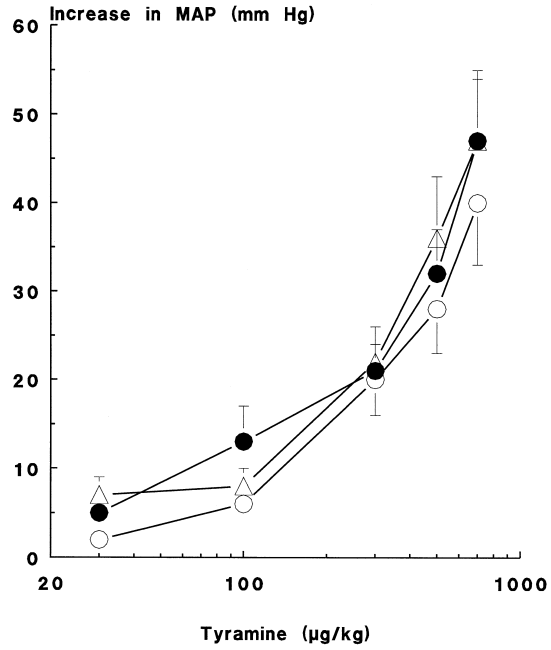


Fig. 1. Dose-dependent increase in mean arterial pressure (MAP) in diabetic rats and in non-diabetic controls after administration of tyramine (30, 100, 300, 500 and 700 μg/kg). The MAP response to tyramine of the Org 2766-treated diabetic rats (△, $n = 7$), of the placebo-treated diabetic rats (○, $n = 8$) and of the non-diabetic controls (●, $n = 8$) was not significantly different between the three groups.

interval between the highest dose of tyramine and the lowest dose of phenylephrine.

Sciatic nerve blood flow was measured by laser-Doppler flowmetry in urethane-anaesthetized rats. In recent years laser-Doppler flowmetry has become the accepted method for the measurement of nerve blood flow. Our group has published several reports on the use of this technique (Kappelle et al., 1993, 1994a; Van Buren et al., 1996). In short, blood flow in the left sciatic nerve was assessed using a laser-Doppler flowmeter (Periflux PF3, Perimed, Sweden, Perimed needle probe PF302, tip diameter of 0.45 mm) and was expressed in arbitrary units (perfusion units, PU). Basal nerve blood flow was determined by taking 14 consecutive flow measurements at different locations on the nerve (Yasuda et al., 1989; Van Buren et al., 1996).

### 2.4. Data acquisition and analysis

Arterial blood pressure and heart rate were measured by means of a Viggo-Spectramed DTX/plus transducer connected to a preamplifier/biotachometer system (University of Utrecht). Femoral blood flow was measured with a Transonic T206 2-channel small animal blood flowmeter (Transonic Systems, Ithaca, NY, USA). All data were processed with a Bio Signal Processing System (Instrumental Services, University of Limburg, Maastricht, Netherlands) and a Wekagraph WK-821 AR recorder. The collected data were processed with a Compaq Deskpro 386/20MHz computer, and mean arterial blood pressure and femoral blood flow were determined once per 500 ms. Mean arterial blood pressure was calculated from the formula: MAP = (2 × diastolic blood pressure + systolic blood pressure)/3.

### 2.5. Outline of the study

A group of 30 rats was randomly divided into three subgroups of 10. Group 1: non-diabetic and food-restricted control rats were fed on a food-restricted diet for 12 weeks. Group 2: diabetic rats treated with Org 2766, 75 μg/kg in 0.5 ml saline s.c. every 48 h. Group 3: diabetic

Table 2
Baseline haemodynamic variables

| Group | $n$ | $P_{sys}$ (mmHg) | $P_{dias}$ (mmHg) | HR (bpm) | FF (ml/min) | NBF (PU) |
|---|---|---|---|---|---|---|
| Diet | 8 | 141 ± 7 | 92 ± 7 | 380 ± 19 | 2.28 ± 0.25 | 56 ± 2 |
| DMOrg | 7 | 129 ± 9 | 82 ± 8 | 346 ± 20 | 1.52 ± 0.30 [a] | 28 ± 1 [a] |
| DMPla | 8 | 136 ± 6 | 90 ± 8 | 366 ± 17 | 1.32 ± 0.30 [a] | 31 ± 1 [a] |

Basal values (means ± S.E.M.) for the number of animals ($n$) for systolic pressure ($P_{sys}$), diastolic pressure ($P_{dias}$), heart rate (HR; bpm, beats/min), femoral blood flow (FF) and sciatic nerve blood flow (NBF; PU, perfusion units), at week 12, of the non-diabetic group (Diet), the Org 2766-treated diabetic group (DMOrg) and the placebo-treated diabetic group (DMPla). [a] Statistically significant difference from non-diabetic controls, $P < 0.05$.

rats treated with placebo, 0.5 ml saline s.c. every 48 h. Treatments (groups 2 and 3) were started 6 weeks after the streptozotocin injection, when experimental diabetic neuropathy was manifest (Bravenboer et al., 1993), and lasted 6 weeks (Van Buren et al., 1996). After 12 weeks all rats were prepared for measurements of blood pressure and femoral blood flow. Adrenergic response was assessed by making dose-response curves for the effect of i.v. tyramine and phenylephrine. In a separate experiment a group of rats was randomly divided into three subgroups as de-

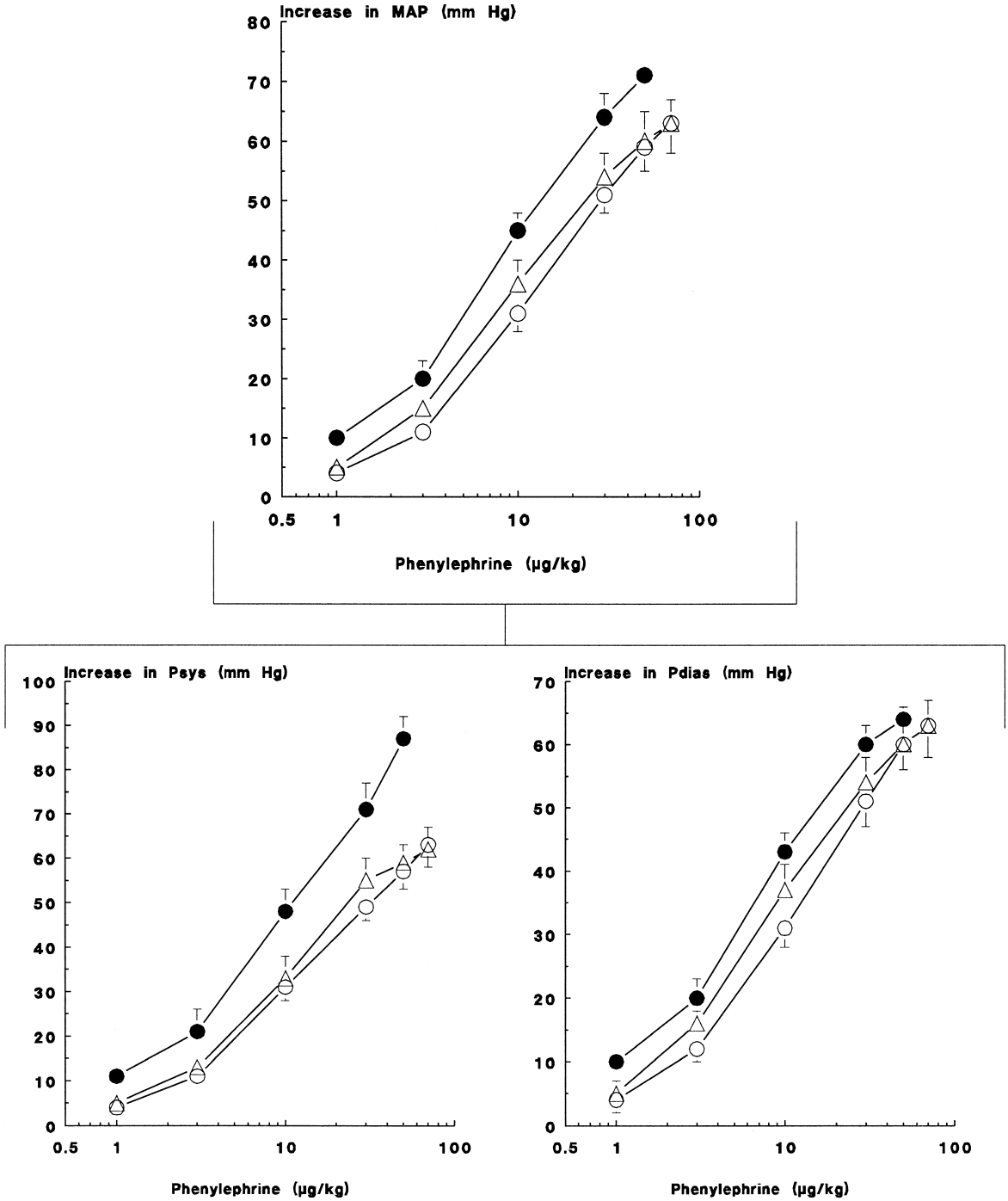


Fig. 2. Dose-dependent increase in mean arterial pressure (MAP) in diabetic rats and in non-diabetic controls after administration of phenylephrine (1, 3, 10, 30, 50 and 70 µg/kg). The MAP response to phenylephrine of the placebo-treated diabetic rats (○, $n = 8$) was significantly reduced compared to the response of the non-diabetic controls (●, $n = 8$) ($F(1,14) = 14.40$, $P < 0.05$). The MAP response was the same in the Org 2766-treated diabetic rats (△, $n = 7$) and in the placebo-treated diabetic rats. The insets show the dose-dependent increase in systolic pressure ($P_{sys}$) and diastolic pressure ($P_{dias}$). The $P_{sys}$ response to PHE was smaller in the placebo-treated diabetic rats than in the non-diabetic control rats ($F(1,14) = 19.51$, $P < 0.05$), as was the $P_{dias}$ response ($F(1,14) = 7.79$, $P < 0.05$).

scribed above. After 12 weeks, the sciatic nerve blood flow was measured by laser-Doppler blood flowmetry.

### 2.6. Data analysis

The data are presented as means ± standard errors of the mean (S.E.M.). All measurements were carried out in a blind fashion. Differences in baseline haemodynamic variables between the groups were assessed by analysis of variance (ANOVA), followed by Student-Newman-Keuls tests to assess the significance of differences between pairs of groups. Group differences in adrenergic responsiveness were tested with a multivariate analysis of variance with repeated measurements (MANOVA). Statistical significance was assumed at the 0.05 level.

## 3. Results

### 3.1. Body weights and plasma glucose levels

All rats that received streptozotocin became diabetic. The blood glucose levels remained > 15 mmol/l throughout the study (Table 1). The mean body weights of the non-diabetic rats and of the diabetic rats are given in Table 1.

### 3.2. Baseline haemodynamic variables

Baseline mean arterial pressure, heart rate and femoral blood flow were determined 15 min after they had stabilized. The data on basal haemodynamic variables are summarized in Table 2. The mean arterial pressure of the placebo-treated and that of the Org 2766-treated group were not significantly different from the basal mean arterial pressure of the food-restricted control group (MAP: Diet 108 ± 7; DMOrg 98 ± 8; DMPla 105 ± 7 mmHg). The femoral blood flow and sciatic nerve blood flow were reduced by approximately 42% ($P < 0.05$) and 48% ($P < 0.05$), respectively, and there was no difference in the baseline haemodynamic variables for the Org 2766-treated diabetic rats and those for the placebo-treated diabetic rats after 6 weeks of treatment.

### 3.3. Tyramine- and phenylephrine-induced cardiovascular effects

There was no significant difference in the dose-dependent increase in blood pressure elicited by tyramine between the non-diabetic control group, the placebo-treated diabetic group and the Org 2766-treated diabetic group (Fig. 1). The diabetic rats showed hyporesponsiveness to phenylephrine as compared to the non-diabetic controls ($F(1,14) = 14.40$, $P < 0.05$). After phenylephrine, systolic blood pressure increased less in the placebo-treated diabetic group than in the non-diabetic control group ($F(1,14) = 19.51$, $P < 0.05$), whereas the response of systolic blood pressure to phenylephrine was the same in the Org 2766-treated diabetic group and in the placebo-treated diabetic group. The increase in diastolic blood pressure elicited by phenylephrine was also smaller in the placebo-treated diabetic group than in the non-diabetic control group ($F(1,14) = 7.79$, $P < 0.05$) (Fig. 2).

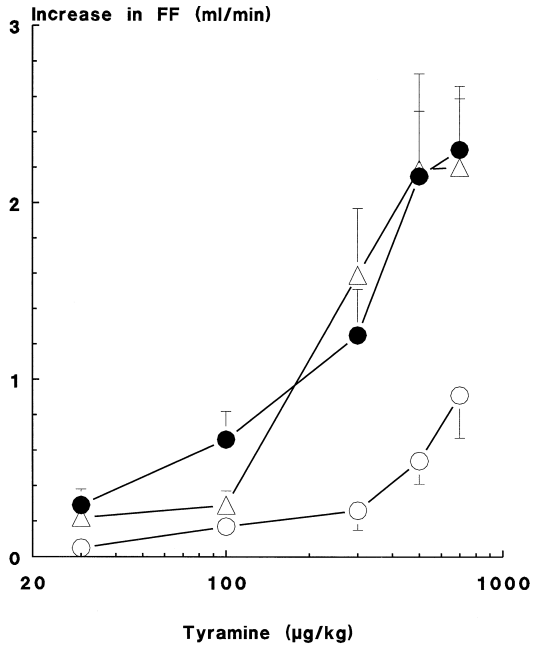


Fig. 3. Dose-dependent increase in femoral blood flow (FF) in diabetic rats and in non-diabetic controls after administration of tyramine (30, 100, 300, 500 and 700 µg/kg). The FF response to tyramine of the placebo-treated diabetic rats (○, $n = 8$) was significantly reduced compared to the response of the non-diabetic controls (●, $n = 8$) ($F(1.13) = 20.21$, $P < 0.05$). The response to tyramine of the Org 2766-treated diabetic rats (△, $n = 7$) was significantly increased compared to the response of the placebo-treated diabetic rats ($F(1,13) = 13.99$, $P < 0.05$) and not different from the response of the non-diabetic controls.

The femoral blood flow response to tyramine of the placebo-treated diabetic rats was smaller than that of the non-diabetic controls ($F(1,13) = 20.21$, $P < 0.05$) and that of the Org 2766-treated diabetic rats ($F(1,13) = 13.99$, $P < 0.05$). The femoral blood flow response to tyramine of the Org 2766-treated diabetic rats was similar to that of the non-diabetic control rats (Fig. 3). In doses up to 10 µg/kg, phenylephrine increased femoral blood flow less in the placebo-treated diabetic rats than in the non-diabetic controls ($F(1,14) = 9.94$, $P < 0.05$) or in the Org 2766-treated diabetic rats. Phenylephrine elicited a similar change in femoral blood flow in Org 2766-treated diabetic rats and in non-diabetic control rats, and at higher doses the femoral

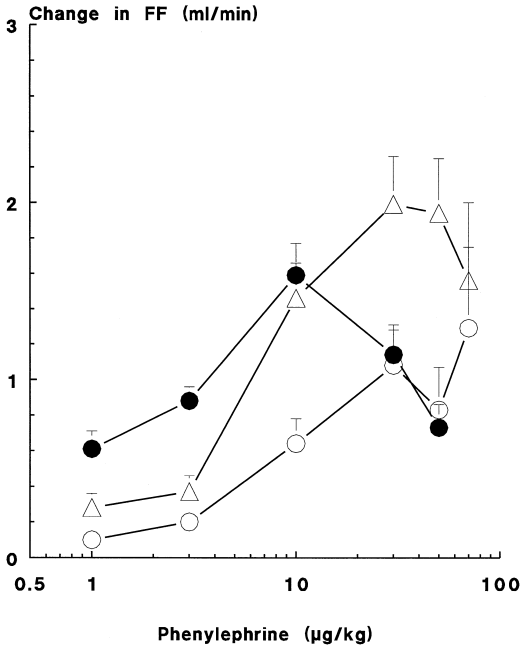


Fig. 4. Change in femoral blood flow (FF) in diabetic rats and in non-diabetic controls after administration of phenylephrine (1, 3, 10, 30, 50 and 70 μg/kg). The FF response to phenylephrine of the placebo-treated diabetic rats (○, $n = 8$) was significantly reduced at doses up to 10 μg/kg compared to the response of the non-diabetic controls (●, $n = 8$) ($F(1,14) = 9.94$, $P < 0.05$). The response to phenylephrine of the Org 2766-treated diabetic rats (△, $n = 7$) was increased compared to the response of the placebo-treated diabetic rats and was shifted to the right compared to the response of the non-diabetic controls.

blood flow response was even greater in the Org 2766-treated rats (Fig. 4).

## 4. Discussion

Autonomic neuropathy as a complication of diabetes mellitus can have serious consequences for the functioning of the cardiovascular system either in the heart (Jackson and Carrier, 1983; Chang and Lund, 1986) or in the micro- and macrocirculation (Lucas, 1985; Zatz and Brenner, 1986; Hill and Larkins, 1989; Stevens et al., 1991).

This study demonstrated a strong decrease in the microcirculatory sciatic nerve blood flow in the diabetic animals (Table 2). Similar reductions in nerve blood flow, after 1 to 4 months of streptozotocin-induced diabetes in rats, have also been found by others using different measuring techniques (Cameron et al., 1991; Stevens et al., 1994). Since basal systemic blood pressure was similar in our experimental groups, it is suggested that changes in sciatic nerve blood flow might be due to local vasomotor abnormalities in diabetes. These abnormalities could include impairment of the microvasculature by microangiopathy (Zatz and Brenner, 1986; King et al., 1993). Earlier work from our group (Kappelle et al., 1993, 1994a; Van Buren et al., 1996) does suggest that reduced nerve blood flow is a possible consequence of impaired sympathetic innervation of the vasa nervorum. However, a diminished sympathetic innervation of the vasa nervorum would have led to a reduced $\alpha$-adrenoceptor-mediated vasoconstriction, and therefore to an increased nerve blood flow. Therefore, the question arises as to how a reduced sciatic nerve blood flow can be explained by a dysfunctional sympathetic innervation of the vasa nervorum. Another possible explanation is that disturbances in the macrocirculatory supply to the nerve may also lead to a reduced sciatic nerve blood flow. Nukada et al. (1993) had found that the femoral artery is the most important blood supplying source for maintaining microcirculatory sciatic nerve blood flow. Therefore, in the present study we measured femoral blood flow in addition to sciatic nerve blood flow.

The results presented demonstrated a reduction in both sciatic nerve blood flow (48%) and ipsilateral femoral blood flow (42%) in diabetic animals (Table 2). These results suggest that a reduction in sciatic nerve blood flow might be a consequence of a proportionally reduced femoral blood flow, indicating that a reduced macrocirculatory blood flow can be (partly) responsible for the decrease in nerve blood flow. These results are in line with the suggestions of Riccardi et al. (1988) that there may be an association between retinopathy (microangiopathy) and impaired peripheral arterial circulation (macroangiopathy). Also Kiff et al. (1991) demonstrated, from measurements of hindquarter flow by pulsed Doppler flowmetry in the distal abdominal aorta of rats, that streptozotocin treatment can decrease this flow. On the other hand one must take into account that there are also other vascular beds downstream which modulate femoral flow, e.g., in hindquarter skeletal and skin muscles. So we cannot exclude that alterations in these vascular beds also modulate femoral flow and consequently the related nerve blood flow.

The decrease in femoral blood flow in the diabetic animals might have been a consequence of either vasomotor abnormalities of the femoral artery and/or cardiac dysfunction, e.g., altered cardiac output resulting in an altered regional distribution of blood flow in the diabetic rat (Lucas, 1985; Hill and Larkins, 1989). The vasomotor abnormalities can be ascribed to a local macrovascular pathology, including an altered release and/or effectiveness of nitric oxide (Bucala et al., 1991; King et al., 1993) and endothelin (Takahashi et al., 1990) or changes in the levels or actions of vasoactive hormones, e.g., angiotensin (Katayama and Lee, 1985). Another explanation for the vasomotor abnormalities of the femoral artery might be disturbances in the autonomic, e.g., sympathetic, innervation of the femoral artery. We now studied the involvement of the sympathetic nervous system in the flow-decreasing effect of diabetes by using the approach of Van der Zee et al. (1990) and Kappelle et al. (1993, 1994a,b). Thus the response to phenylephrine was used as a measure of adrenergic postsynaptic function and the response to tyramine as a measure of mainly presynaptic function. The

tyramine-induced increase in femoral blood flow in non-diabetic animals can been fully ascribed to an increase in either perfusion pressure (i.e., MAP; Figs. 1 and 3) or cardiac output due to the release of catecholamines by tyramine. In contrast, the femoral blood flow response to tyramine was strongly reduced in diabetic rats. However, the blood pressure response to tyramine was the same for both groups, suggesting a sympathetic dysregulation of femoral blood flow or an altered cardiac output due to sympathetic dysfunction. This hypothesis, i.e., sympathetic dysfunction, is supported by the results obtained with Org 2766: Org 2766 restored the femoral blood flow response to tyramine to that of the control rats. This ameliorating effect can only be ascribed to neuroprotective effects (Gispen, 1990) and not to haemodynamic actions of Org 2766, since this ACTH-(4–9) analogue lacks a direct effect on the cardiovascular system in rats (De Wildt et al., 1993), and, as far as we know, there is no evidence for any effect of Org 2766 on catecholamine release, synthesis or metabolism. This is consistent with the observation that the responsiveness to noradrenaline within the cardiovascular system is decreased in diabetes mellitus, and can be restored by Org 2766 (Van der Zee et al., 1990; Kappelle et al., 1994b). Additionally, a diminished femoral blood flow response to tyramine can be of cardiac origin. Sundaresan et al. (1984) reported a decrease in the number of cardiac $\beta$-adrenoceptors after streptozotocin treatment. This could lead to cardiac ($\beta$) adrenergic hyporesponsiveness and therefore to alterations in cardiac output in diabetic rats (Lucas, 1985; Hill and Larkins, 1989). Thus an altered tyramine response in the heart could have led to a diminished femoral blood flow via a reduction of cardiac output. For this reason, neuronal protection of cardiac sympathetic fibres by Org 2766 could be an alternative explanation for the restoration of the tyramine-mediated femoral blood flow response.

The blood pressure response to phenylephrine was smaller in diabetic rats than in non-diabetic rats, mainly as a consequence of an effect on systolic blood pressure (Fig. 2). The difference in the blood pressure response to tyramine (no hyporesponsiveness) and phenylephrine (hyporesponsiveness) was rather surprising but can be explained by a diabetes-induced cardiac dysfunction. In fact, the decreased response to phenylephrine can be ascribed to an increased afterload, leading to further impairment of the function of the myocardium, resulting in a decrease in (systolic) blood pressure. In contrast, tyramine, by releasing catecholamines from presynaptic terminals in the heart, can support cardiac function, relieving the increase in afterload due to its vasoconstrictor action. Phenylephrine elicited a biphasic femoral blood flow response in the non-diabetic control rats (Fig. 4). The increase in femoral blood flow was caused passively through the dose-dependent increase in perfusion pressure or cardiac output. The fall in femoral blood flow seen at phenylephrine doses higher than 10 $\mu$g/kg may be explained by local vasoconstriction of the femoral artery (bed) followed by an increase in vascular resistance and/or reduction in cardiac output. In the diabetic rats the femoral blood flow response to phenylephrine was strongly reduced, which could have been a consequence of either disturbed local postsynaptic function (Kamata et al., 1989) or a phenylephrine-evoked decrease in cardiac output (see above) and hence in femoral blood flow. Org 2766 restored the femoral blood flow response to phenylephrine in diabetic rats. As Org 2766 has neuroprotective effects (Gispen, 1990) but no direct cardiovascular effects (De Wildt et al., 1993), it is unlikely that Org 2766 had an effect on the postsynaptic defects in diabetic rats (Van Buren et al., 1996). It is more likely that Org 2766 had an ameliorating effect on the cardiac sympathetic innervation (Sundaresan et al., 1984; Chang and Lund, 1986), which would lead to an increase in cardiac function (cardiac output) and therefore to an improved response to phenylephrine of the femoral artery of diabetic rats.

There was no difference between the basal haemodynamic variables, e.g., blood pressure, femoral and nerve blood flow, of the Org 2766-treated diabetic rats and those of non-diabetic rats. However, Org 2766 restored the disturbed adrenergic responsiveness of the femoral artery to its non-diabetic level. This suggests that these disturbances have little, if any, effect on the reduced basal femoral blood flow of diabetic rats. The same effect was seen for the sciatic nerve blood flow (Van Buren et al., 1996).

In conclusion, the decrease in basal femoral blood flow in diabetic rats might have resulted from vasomotor abnormalities and/or cardiac dysfunction. We cannot exclude the possibility that macrovascular changes within the femoral artery due to diabetes in combination with a disturbed sympathetic tone regulation of this artery also can play a role in the reduced femoral blood flow. This decrease in femoral blood flow might be (partly) responsible for the lowered sciatic nerve blood flow. Therefore, the decrease in sciatic nerve blood flow is the consequence, not only of local abnormalities (microangiopathy and/or altered nervi vasorum), but also of an impaired peripheral blood circulation. Adrenergic stimulation with tyramine and phenylephrine indicated that there is sympathetic damage in diabetic neuropathy. Whether this sympathetic damage in diabetic rats is of local origin or of cardiac origin is not clear from the results of this study. Further studies will investigate the cardiac sympathetic innervation in diabetic rats. Finally, adrenergic disturbances of femoral blood flow play only a small role in the reduced basal femoral blood flow of the streptozotocin-diabetic rats.

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