Beneficial effect of the Ca^{2+} antagonist, nimodipine, on existing diabetic neuropathy in the BB/Wor rat

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1 Neuropathy is a frequently diagnosed complication of diabetes mellitus. Effective pharmacotherapy is not available.

2 The spontaneously diabetic BB/Wor rats develop secondary complications like neuropathy as do human diabetic patients.

3 BB/Wor rats treated with insulin via a subcutaneous implant show a significant impairment of sensory and motor nerve conduction velocity 6 weeks after the onset of diabetes mellitus.

4 Intraperitoneal treatment of diabetic BB/Wor rats with the Ca^{2+} antagonist, nimodipine (20 mg kg^{-1}), from week 6 onwards every 48 h for a period of 6 weeks resulted in a significant increase of sensory and motor nerve conduction velocity.

5 Twelve weeks after the onset of diabetes mellitus BB/Wor rats show a 40% impairment of sciatic nerve blood flow as compared to the non-diabetic age-matched controls. Treatment with nimodipine (20 mg kg^{-1}) from week 6 onwards significantly increased the sciatic nerve blood flow as compared to placebo-treated diabetic BB/Wor rats.

6 The adrenergic responsiveness of the vasa nervorum of the sciatic nerve to tyramine and phenylephrine was investigated as a parameter for autonomic neuropathy.

7 The fact that nimodipine treatment restored the reduced response to tyramine independently of the reduced postsynaptic phenylephrine responsiveness indicates that nimodipine improves adrenergic responsiveness mainly at the presynaptic level.

Keywords: Diabetes mellitus; neuropathy; nerve conduction velocity; nerve blood flow; adrenergic responsiveness; peripheral nerve regeneration; Ca^{2+} antagonist; nimodipine

Introduction

Patients with diabetes mellitus may suffer from a number of chronic complications. It remains to be determined to what extent chronic hyperglycaemia itself is responsible for these complications. With respect to diabetic neuropathy, Pirart (1978) has demonstrated that the prevalence of this complication is increased in patients with poor metabolic control. Although maintenance of normoglycaemia may certainly have a beneficial effect on diabetic complications such as the neuropathy, the pathogenesis of these complications seems to involve additional factors (Strowig & Raskin, 1992). A major focus of research is the prevention and treatment of complications such as neuropathy. The somatic and autonomic nervous system is affected during diabetes mellitus, resulting in functional disturbances and clinical morbidity (Thomas & Eliasson, 1984; Horowitz *et al.*, 1986; Watkins, 1990; Smout *et al.*, 1991).

Most of our current knowledge on diabetic neuropathy is based on the results of animal studies. The streptozotocin (STZ)-induced diabetic rat is the most frequently used model to study experimental diabetes mellitus (Mayer & Tomlinson, 1983). The spontaneously diabetic BB/Wor rat, however, mimics human diabetes mellitus in a superior way and has proved to be a promising animal model of insulin-dependent diabetes mellitus (IDDM) (Mordes *et al.*, 1987; Crisá *et al.*, 1992).

Diabetic BB/Wor rats gradually develop secondary complications as do human patients with diabetes mellitus. Complications such as pathological changes in the retina (Fitzgerald & Caldwell, 1990), kidneys (Cohen *et al.*, 1987), nerves (Sima & Thibert, 1982; Sima *et al.*, 1990; Zhang *et al.*, 1990) have been reported.

We used the BB/Wor rat to study the pathogenesis of diabetic neuropathy and to determine the effect of the Ca^{2+} antagonist, nimodipine, on the affected nervous system. Nimodipine (isopropyl-(2-methoxy-ethyl)-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinic dicarboxylate) is an L-type Ca^{2+} channel blocker (Scriabine *et al.*, 1989) which exerts profound neuropharmacological effects (Traber & Gispen, 1989). Recently, we demonstrated beneficial effects of nimodipine on nerve conduction velocity in STZ-induced diabetic rats (Kappelle *et al.*, 1992; 1993b).

Decreased nerve blood flow (NBF) appears to be a crucial factor in the pathogenesis of diabetic neuropathy (Cameron *et al.*, 1991; Hotta *et al.*, 1992); therefore the question was raised whether vascular effects of nimodipine (Betz *et al.*, 1985) contribute to its ameliorative effect in experimental diabetic neuropathy (Kappelle *et al.*, 1993a). In addition to NBF, blood flow control mechanisms are an important issue in the study of diabetic neuropathy. In the present study, we investigated the influence of diabetes mellitus and the effect of nimodipine treatment on three targets: sensory and motor nerve conduction velocity, NBF and autonomic sympathetic function of the nervi vasorum. In this way we attempted to obtain insight into the functioning of the peripheral somatic and autonomic nerve and the vasa nervorum of the diabetic BB/Wor rat and the mechanism of action of nimodipine in experimental diabetic neuropathy.

Methods

Animals

Twenty-five BB/Wor rats and 12 WB/Prone, non-diabetic, age-matched control rats were obtained from the Møllengard

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Institute, Skensved, Denmark. The rats were housed on sawdust in macrolon cages, 2 rats per cage and maintained on a 12:12 h light-dark cycle with food and water *ad libitum*. Insulin treatment was started immediately after the development of hyperglycaemia (blood glucose $> 15 \text{ mmol l}^{-1}$) ($n = 22$). Since continuous subcutaneous insulin infusion in human diabetics has received more and more attention, slow release insulin implants were used instead of daily subcutaneous injections. Implants (Møllengard Institute, Skensved, Denmark), containing 17% bovine insulin (0.5% Zn) and 83% palmitic acid, weight about 26 mg and dimensions of 2 mm in diameter and 7 mm in length were placed subcutaneously. Slow surface erosion of the implant *in vivo* gradually releases the entrapped insulin at a rate of 2 u per day for more than 40 days. The implant was removed after 40 days and a new implant was inserted. Body weight was measured every week. Blood glucose was controlled every third week. Blood samples were obtained from the tail vein. Blood glucose was monitored with a Minilab (Bayer GmbH, Munich, Germany).

Electrophysiology

Sensory nerve conduction velocity (SNCV) and motor nerve conduction velocity (MNCV) were measured every 2 weeks from the start of the treatment. The method used has been described in detail by De Koning & Gispen (1987). In short, the sciatic and tibial nerves were stimulated at the sciatic notch and the ankle, respectively, by monopolar needle electrodes. Responses were recorded from the small muscles of the foot by means of surface electrodes. SNCV and MNCV were calculated from the latencies of these responses and the distance between the two stimulation points.

Nerve blood flow measurements

The rats were anaesthetized with 10% urethane, 1.2 ml 100 g^{-1} , i.p., and placed on a homiothermic heating blanket. Rectal temperature was maintained at $\pm 37^\circ\text{C}$. Urethane was used instead of barbiturates in order to ensure minimal anaesthetic interference with the cardiovascular system and to leave autonomic reflexes intact. The left sciatic nerve was exposed and the fascia surrounding the nerve was carefully removed. The nerve was positioned on a perspex holder just at the place where the nerve emerges from the musculus gluteus maximus. The part of the nerve positioned on the holder was cleaned of remaining fascia tissue, disturbing the epineurial blood vessels as little as possible. The sciatic nerve was kept moist with saline solution at 37°C . Blood flow was monitored with a laser-Doppler flowmeter (Perimed Sweden, Periflux PF3). The flowmeter was equipped with a 2 mW Helium-Neon laser with a wave length of 632.8 nm. Flow is expressed in arbitrary units (perfusion units (PU)). The low pass filter was set at a cut-off frequency of 4 kHz. The laser probe (Perimed needle probe PF302, tip diameter of 0.45 mm) was positioned on the nerve just contacting the saline film covering the nerve. The flow signal was averaged at 0.2 s periods. Care was taken to avoid placing the probe in the proximity of larger blood vessels. Basal sciatic NBF was determined by taking the average of 10 out of 14 consecutive flow measurements at different locations on the nerve (the two lowest and two highest values were excluded). At the end of the experiment the background LDF value was assessed by monitoring the LDF output signal after the rat had been killed with i.v. administered pentobarbitone. Background values were subtracted from the values obtained during the experiment.

Assessment of nervi vasorum sympathetic autonomic function

LDF allows the continuous measurement of microvascular blood flow. This makes this technique especially suitable for

monitoring rapid flow changes resulting from adrenergic stimulation. The response to phenylephrine was used as a measure of adrenergic postsynaptic function. The NBF response to tyramine is a result of combined pre- and postsynaptic effects. A polyethylene catheter, containing 50 iu ml^{-1} sodium heparin in isotonic saline, was implanted in the right common iliac artery, its tip positioned just at the bifurcation of the abdominal aorta, after the basal sciatic NBF was quantified. The right artery was closed around the catheter with ligatures, to allow all vasoactive agents applied via the catheter to end up in the left common iliac artery. The arterial catheter was connected to a pressure transducer (Viggo-Spectramed disposable DTX/plustm) via a Y-shaped injection system. Vasoactive agents could be administered locally through this system to the left hind paw of the animal, without interruption of blood pressure recording.

Responses of sciatic NBF to phenylephrine (1, 2 and $3 \mu\text{g kg}^{-1}$) and tyramine (75, 150 and $300 \mu\text{g kg}^{-1}$) were recorded in each animal. The number of doses was rather limited because of an irreversible decline in responsiveness after more than 10 doses (Zochodne & Low, 1990). Blood pressure is expressed as mean arterial pressure (MAP) in mmHg ($\text{MAP} = (\text{systolic pressure} + 2 \times \text{diastolic pressure})/3$).

Phenylephrine and tyramine had minor effects on MAP. Therefore, the responses to phenylephrine and tyramine are expressed as '% flow change'. This measure was calculated by correcting the change in flow for effects on MAP, since minor changes in MAP are followed passively by NBF (Sundqvist *et al.*, 1985).

$$\% \text{ flow change} = \frac{\text{NBF}_{\text{post}} * (\text{MAP}_{\text{pre}} / \text{MAP}_{\text{post}}) - \text{NBF}_{\text{pre}}}{\text{NBF}_{\text{pre}}} * 100\%$$

Drugs

Nimodipine 20 mg kg^{-1} (a gift from Bayer AG, Elberfeld, Germany) as a micronized powder was dissolved in polyethylene glycol (PEG 400) 1 ml kg^{-1} under dim light and injected intraperitoneally (i.p.) as a freshly prepared solution.

Phenylephrine, an α -adrenoceptor agonist ((-)-phenylephrine hydrochloride, Sigma, St. Louis, MO, U.S.A.), was dissolved in saline (0.9%) at doses of 1, 2 and $3 \mu\text{g kg}^{-1}$ respectively and tyramine, which releases endogenous nor-adrenaline from presynaptic terminals (tyramine hydrochloride, Regis Chemical Co., Chicago, IL, U.S.A.), was dissolved in saline (0.9%) at doses of 75, 150 and $300 \mu\text{g kg}^{-1}$. The drugs were administered i.v. at a rate of $200 \mu\text{l min}^{-1}$ with an infusion pump.

Data analysis

Data are presented as means $\pm$ standard error of the mean (s.e.mean), unless indicated otherwise. Experiments were carried out in a blind fashion. The treatment code was partially broken at the end of the experiment to allow analysis of data for the various treatment groups. The results of SNCV, MNCV and adrenergic responsiveness were analysed by multivariate analysis of variance for repeated measurements (MANOVA) followed by *t* tests. Group differences in NBF were assessed by an analysis of variance (ANOVA) followed by *t* tests. The correlation between changes in NBF and SNCV/MNCV was determined by regression analysis, using the NCV values of week 12. The final treatment code was broken only after the analysis of the data was completed.

Outline of the study

Six weeks after the development of diabetes mellitus the rats were randomly divided into two groups. Group 1 (nimodipine, $n = 11$) received nimodipine, 20 mg kg^{-1} dissolved in PEG i.p. every 48 h. Group 2 (placebo, $n = 11$) received PEG, 1 ml kg^{-1} i.p. every 48 h. Group 3 consisted of non-diabetic WB/prone rats (control, $n = 12$), receiving PEG

Table 1 Mean blood glucose levels (Gluc) (mmol l⁻¹) at wk 0 (onset of diabetes mellitus), wk 6 (start treatment) and wk 12 (end of the experiment), body weight (BW) in (g) at wk 0 and wk 12, and systolic (P_{syst}) and diastolic pressure (P_{diast}) at wk 12 (mmHg) $\pm$ s.e.mean of the non-diabetic controls, the placebo-treated diabetic rats and the nimodipine-treated diabetic rats.

	Gluc wk 0	Gluc wk 6	Gluc wk 12	BW wk 0	BW wk 12	P _{syst} wk 12	P _{diast} wk 12
Control	6.7 (1.0)	5.9 (0.9)	6.3 (1.1)	292 (4)	385 (7)	118 (5)	91 (4)
Placebo	23 (1.1)*	21 (1.1)*	18 (1.0)*	274 (4)	313 (5)*	115 (6)	87 (7)
Nimodipine	23 (1.1)*	23 (2.3)*	18 (0.9)*	279 (4)	306 (6)*	121 (5)	89 (6)

* $P < 0.001$ as compared to control.

1 ml kg⁻¹ i.p. every 48 h. SNCV and MNCV were measured every 2 weeks from the start of treatment. After 6 weeks of treatment (12 weeks after the development of diabetes mellitus) sciatic NBF was assessed by laser-Doppler flowmetry. Thereafter the response of sciatic NBF to local application of tyramine and phenylephrine was tested.

Results

Blood glucose, body weight and blood pressure

The glucose levels of 22 rats increased to levels > 15 mmol l⁻¹ and remained stable throughout the study (Table 1). The mean body weight of the non-diabetic control rats and of the diabetic rats increased during the experiment (Table 1). Neither systolic nor diastolic blood pressure differed significantly among the three experimental groups at the end of the experiment (Table 1).

Sensory and motor nerve conduction velocity

SNCV and MNCV of the age-matched controls showed an increase until week 6 and remained relatively stable thereafter. The diabetic rats showed a smaller increase in SNCV and MNCV. The difference in SNCV and MNCV between the diabetic and non-diabetic rats reached significance within 6 weeks. Over the treatment period placebo-treated diabetic rats had significantly impaired SNCV and MNCV as compared to the non-diabetic, age-matched controls (SNCV: $F(1,21) = 243.5$, $P < 0.001$; MNCV: $F(1,21) = 172.3$, $P < 0.001$). Treatment with nimodipine resulted in an increase in SNCV and MNCV as compared to the placebo treatment (SNCV: $F(1,20) = 40.63$, $P < 0.001$; MNCV: $F(1,20) = 18.20$, $P < 0.001$) (Figure 1). Nimodipine treatment prevented a further increase in the diabetic SNCV and MNCV deficit. However, nimodipine was not able to restore the deficit to the level of the age-matched, control rats (SNCV: $F(1,21) = 137.77$, $P < 0.001$; MNCV: $F(1,21) = 197.5$, $P < 0.001$) (Figure 1). Throughout the experimental period there was no significant difference in body weight between the nimodipine-treated and the placebo-treated diabetic rats (Table 1). Blood pressure was measured just before vas nervorum adrenergic responsiveness was measured and did not significantly differ between the three groups (Table 1).

Measurement of sciatic nerve blood flow

Sciatic NBF was measured 12 weeks after the development of diabetes mellitus. At this time, the diabetic rats had been treated with nimodipine or placebo for 6 weeks. The sciatic NBF of the non-diabetic controls was significantly higher than the sciatic NBF of the placebo-treated diabetic rats ($P < 0.001$) (Figure 2). Treatment with nimodipine significantly ameliorated the deficit observed in the diabetic rats ($P < 0.001$) but could not completely restore the deficit to the level of the age-matched controls ($P < 0.001$) (Figure 2). Furthermore, NBF significantly correlated with nerve con-

Figure 1 Motor (a) and sensory (b) nerve conduction velocity: treatment started 6 weeks after the development of diabetes mellitus. Over the treatment period placebo-treated diabetic rats (Δ , $n = 11$) had significantly impaired SNCV and MNCV as compared to the non-diabetic age-matched controls (O , $n = 12$) (SNCV: $F(1,21) = 243.5$, $P < 0.001$; MNCV: $F(1,21) = 172.3$, $P < 0.001$). Chronic nimodipine treatment of BB/Wor, diabetic rats ($\blacksquare$, $n = 11$) significantly ameliorated the deficit observed in placebo-treated rats after 12 weeks of diabetes mellitus (SNCV: $F(1,20) = 40.63$, $P < 0.001$; MNCV: $F(1,20) = 18.20$, $P < 0.001$) but could not completely restore the deficit as compared to the non-diabetic, age-matched control group (SNCV: $F(1,21) = 137.77$, $P < 0.001$; MNCV: $F(1,21) = 197.5$, $P < 0.001$).

duction velocities in the animal studied (Figure 3; MNCV $\times$ NBF: $R = 0.81$, $P < 0.001$; SNCV $\times$ NBF: $R = 0.78$, $P < 0.001$).

Measurement of nervi vasorum sympathetic autonomic function

The response of sciatic NBF to locally applied phenylephrine and tyramine was significantly smaller in the placebo-treated diabetic group as compared to the age-matched con-

Figure 2 Sciatic nerve blood flow (NBF) $\pm$ s.e.mean 12 weeks after the development of diabetes mellitus. Treatment started 6 weeks after the development of diabetes mellitus. ANOVA revealed significant differences between the three treatment groups ($F(2,31) = 54.29$, $P < 0.001$). Placebo-treated rats ($n = 11$) showed a significant decrease in NBF as compared to the non-diabetic, age-matched control rats ($n = 12$) ($P < 0.001$). Chronic nimodipine treatment ($n = 11$) of BB/Wor diabetic rats significantly ameliorated the deficit observed in placebo-treated rats after 12 weeks of diabetes mellitus ($P < 0.001$) but could not completely restore the deficit as compared to the non-diabetic, age-matched control group ($P < 0.001$).

Figure 3 Correlation between nerve blood flow (NBF) and motor nerve conduction velocity (MNCV) and sensory nerve conduction velocity (SNCV) of the age-matched control rats, the nimodipine-treated diabetic rats and the placebo-treated diabetic rats, 12 weeks after the induction of diabetes mellitus. $MNCV = 34.79 + 0.53 \times NBF$. $R = 0.81$, $F(1,28) = 121$, $P < 0.001$. $SNCV = 38.40 + 0.46 \times NBF$. $R = 0.78$, $F(1,28) = 100$, $P < 0.001$. (▲ = age-matched control, $n = 11$; ● = diabetes placebo, $n = 9$; ■ = diabetes + nimodipine, $n = 10$).

trols (phenylephrine: $F(1,18) = 36.26$, $P < 0.001$; tyramine: $F(1,18) = 6.92$, $P < 0.05$) (Figure 4). Chronic nimodipine treatment did not significantly affect the response to phenylephrine. However, the response to tyramine was significantly increased compared to that of the placebo-treated

Figure 4 Response of the sciatic nerve blood flow (NBF) to local tyramine (a) (75, 150 and $300\ \mu g\ kg^{-1}$) and to local phenylephrine (b) (1, 2 and $3\ \mu g\ kg^{-1}$) application. The response of the sciatic NBF to local tyramine and phenylephrine administration in the placebo-treated diabetic rats (Δ , $n = 10$) was significantly smaller than in the age-matched control rats ($\bigcirc$, $n = 10$), (tyramine: $F(1,18) = 6.92$, $P < 0.05$; phenylephrine: $F(1,18) = 36.26$, $P < 0.001$). Chronic nimodipine treatment in diabetic rats ($\blacksquare$, $n = 10$) did not increase the response to phenylephrine administration as compared to the placebo treatment in the diabetic rats but significantly increased the response to tyramine as compared to placebo treatment in diabetic rats ($F(1,18) = 6.17$, $P < 0.05$).

diabetic group ($F(1,18) = 6.17$, $P < 0.05$). The response to tyramine in the nimodipine-treated diabetic group was not significantly different from the response of the age-matched controls (Figure 4).

Discussion

Over the years many studies from several laboratories have demonstrated the beneficial effects of different pharmacotherapeutic agents in experimental diabetic neuropathy (Tomlinson, 1992; Bravenboer *et al.*, 1992; 1993). The present study confirms and extends the results of previous STZ-diabetic rat studies (Robertson *et al.*, 1992; Kappelle *et al.*, 1993a,b) in which Ca^{2+} antagonists improved existing experimental diabetic neuropathy.

The beneficial action of nimodipine has been documented in post lesion recovery of function in the peripheral nervous system (Van der Zee *et al.*, 1987; 1991; Bär *et al.*, 1990; Gispen *et al.*, 1991) in the central nervous system (Nyakas *et al.*, 1989), in cisplatin-induced peripheral neuropathy (Hamers *et al.*, 1991) and in counteracting the age-related decline in sensorimotor function and cognition (Schuurman *et al.*, 1987; Gispen *et al.*, 1988; Disterhoft *et al.*, 1989; Van

der Zee *et al.*, 1989). Recently, a preventive effect of nimodipine on the development of STZ-induced diabetic neuropathy was observed (Kappelle *et al.*, 1992). Additional experiments demonstrated that nimodipine ameliorated an existing neuropathy in the STZ-induced diabetic rat (Kappelle *et al.*, 1993b). The beneficial action of nimodipine has been attributed to its direct protective effect on the neurone (Traber & Gispen, 1989; Bär *et al.*, 1990; 1993).

A reduced NBF and associated endoneurial hypoxia in diabetes mellitus have been demonstrated in several studies and are thought to play a role in the pathogenesis of diabetic neuropathy (Tuck *et al.*, 1984; Yasuda *et al.*, 1989; Cameron *et al.*, 1991; Hotta *et al.*, 1992). Furthermore, endoneurial vascular abnormalities have been demonstrated in sural nerve biopsies of diabetic patients (Dyck *et al.*, 1985; Sima *et al.*, 1988; Britland *et al.*, 1990). A putative role of hypoxia in diabetic neuropathy is supported by the fact that longstanding hypoxia *per se* can induce severe damage and slowing of nerve conduction velocity in peripheral nerves (Hendriksen *et al.*, 1992).

Nimodipine, a Ca^{2+} antagonist has relaxing effects on cerebral vessels in animal models (Haws *et al.*, 1983; Towart & Kazda, 1985) and counteracts vasospasms in man (Allen *et al.*, 1983). We measured sciatic NBF in order to clarify whether nimodipine could affect experimental diabetic neuropathy by influencing NBF (Tuck *et al.*, 1984; Low *et al.*, 1987). Nimodipine improved sciatic NBF as compared to the placebo-treated diabetic rats although it could not restore NBF to control levels. It should be noted that chronic nimodipine treatment, in the current dose-regimen, influences neither NBF (data not shown), nor nerve conduction velocity in non-diabetic control rats (Hamers *et al.*, 1991). The improvement of sciatic NBF may well have contributed to the amelioration of experimental diabetic neuropathy, since regression analysis of the data revealed that improvement of NBF correlated with enhanced NCV (Figure 3). The effect of nimodipine on NBF is probably caused by a local vasodilator effect since very little effect on systemic blood pressure was measured. Nimodipine prevents Ca^{2+} influx into vascular smooth muscle (Bevan & Hwa, 1985) leading to a reduced vascular tone (Triggle, 1982; Flaim, 1982) and eventually to an improvement of local blood flow (Flaim & Kanda, 1982). Since vasoconstriction, induced by calcium, is enhanced in diabetes mellitus (White & Carrier, 1990) this could be a factor in the beneficial effect of nimodipine in experimental diabetic neuropathy.

Perhaps the beneficial effect of chronic nimodipine treatment is related to an increased endoneurial capillary density as well, since nimodipine promotes microvessel growth (Yuan *et al.*, 1990). Thus, in addition to direct neuroprotective effects (Traber & Gispen, 1989; Bär *et al.*, 1990; 1993), nimodipine probably has a beneficial effect on the vasa nervorum of the diabetic peripheral nerve.

Several investigators have demonstrated substantial innervation of the vasa nervorum and thus a mechanism of neurogenic control (Amenta *et al.*, 1983; Appenzeller *et al.*,

1984; Renkin, 1984; Milner *et al.*, 1992). Autoregulation of NBF in response to systemic blood pressure changes, as observed in the brain, seems to be negligible (Low & Tuck, 1984; Sundqvist *et al.*, 1985; Monafo *et al.*, 1988; Zochodne & Low, 1990). Little research has been carried out on the nervi vasorum of the diabetic nerve and the results obtained are inconsistent (Dhital *et al.*, 1986; Koistinaho *et al.*, 1990). In the present study a hyporesponsiveness of the vasa nervorum to both tyramine and phenylephrine application was demonstrated in placebo-treated diabetic rats. The hyporesponsiveness to phenylephrine demonstrated postsynaptic defects confirming other observations of decreased noradrenaline responsiveness (Foy & Lucas, 1976; Jackson & Carrier, 1983; Ramos, 1988; Van der Zee *et al.*, 1990) in diabetes mellitus. Increased noradrenaline responsiveness has also been described (White & Carrier, 1990; Morff, 1990). As we are the first to measure noradrenaline responsiveness in the vasa nervorum of diabetic rats, comparison of our results with previous research in other vascular systems is difficult. Since the phenylephrine response is seriously disturbed in diabetic rats it is not possible to quantify presynaptic defects based on the impaired response to tyramine application in diabetic rats.

Chronic nimodipine treatment revealed no difference in the response to phenylephrine administration. This implies that the effect of chronic nimodipine treatment on postsynaptic adrenergic responsiveness if any, is limited. The response to tyramine was restored to the non-diabetic level. These results indicate that nimodipine has a beneficial effect on the presynaptic function of the nervi vasorum of the sciatic nerve of the diabetic rat.

Our results do not allow us to answer all the questions about the mechanism of action of nimodipine in experimental diabetic neuropathy. However, besides its direct neuronal effects and the amelioration of NBF, nimodipine seems to have beneficial effect on the autonomic nerves which innervate the vasa nervorum.

In conclusion, NBF and the neural control of the vasa nervorum are seriously affected in experimental diabetic neuropathy. Nimodipine ameliorated the nerve conduction velocity deficits, the decreased NBF and the decreased vasa nervorum adrenergic responsiveness in BB/Wor diabetic rats. Nimodipine may act at at least three levels; directly on the neurone (Traber & Gispen, 1989; Bär *et al.*, 1990; 1993), on the vasa nervorum and on the nervi vasorum of the affected sciatic nerve. Whether these mechanisms of action depend on the specific blockade of Ca^{2+} influx into the neurone, on direct vascular effects, on long-term effects on microvessel growth (Yuan *et al.*, 1990) or on a combination of these factors remains to be elucidated and is currently under investigation. However, it is clear that the nimodipine is a promising putative pharmacotherapeutic agent against experimental diabetic neuropathy and since other diabetic complications could be ameliorated by Ca^{2+} antagonists (Afzal *et al.*, 1988) it would be interesting to investigate the effects of nimodipine treatment in diabetic patients.

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(Received June 28, 1993
 Revised October 26, 1993
 Accepted November 4, 1993)