Amelioration by the Ca^{2+} antagonist, nimodipine of an existing neuropathy in the streptozotocin-induced, diabetic rat

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1 Neuropathy is a frequently diagnosed complication in diabetic patients but an effective treatment does not exist.

2 The development of neuropathy in streptozotocin-induced diabetic rats was monitored by measuring the motor and sensory nerve conduction velocity in the sciatic nerve.

3 A significant decrease in sensory and motor nerve conduction velocity was apparent in young, 14-week-old diabetic rats as compared to non-diabetic, age-matched controls 4 weeks after the induction of diabetes with streptozotocin.

4 Intraperitoneal treatment with the Ca^{2+} channel blocker, nimodipine, from week 4 onwards, in a dosage of 10 mg kg^{-1} or 20 mg kg^{-1} intraperitoneally per 48 h, resulted in a significant increase in sensory and motor nerve conduction velocity whereas treatment with 5 mg kg^{-1} intraperitoneally per 48 h was not effective.

5 One-year-old, adult, diabetic rats treated with nimodipine 20 mg kg^{-1} (treatment started again 4 weeks after induction of diabetes mellitus) also showed an increase of both sensory and motor nerve conduction velocity as compared to diabetic rats treated with placebo.

6 It is concluded that nimodipine ameliorates existing experimental diabetic neuropathy in streptozotocin-induced diabetic rats in both young and adult animals.

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

Introduction

Although insulin treatment has achieved enormous improvement in the quality of life in diabetic patients, it is unclear whether absolute normoglycaemia will prevent the development of complications such as nephropathy, retinopathy and neuropathy. A major focus of research is the prevention and treatment of these complications from both type 1 and type 2 diabetes mellitus (Pirart, 1978). To date, irrespective of the pathogenesis, there is no treatment of neuropathy other than attempting to decrease the symptoms, such as paraesthesia, pain, muscle weakness and postural hypotension. Over the years numerous studies in experimental diabetic neuropathy (EDN) have been carried out to assess the efficacy of putative pharmacotherapy. Streptozotocin (STZ) is frequently used to destroy the β cells in the islets of Langerhans of rats and so to induce diabetes mellitus (Rerup, 1970). Although neither this nor any other model can mimic the full detail of human diabetes mellitus, the pathology of diabetic neuropathy is close to that observed in man. In EDN, both in the STZ-diabetic rat and in the spontaneously diabetic rat (BB/Wor), several abnormalities such as biochemical, histomorphological and nerve conduction velocity level disturbances have been described (Crisa *et al.*, 1992). Commonly, sensory and motor nerve conduction velocities are greatly decreased in diabetic patients (Thomas & Eliasson, 1975). For this reason, reductions in peripheral sensory and motor nerve conduction velocity (SNCV and MNCV) are frequently taken as indices of EDN and are used to determine the possible effects of pharmacotherapy in rats (Greene *et al.*, 1987).

There are several hypotheses about the pathogenesis of diabetic neuropathy. One hypothesis points to the dysregulation of neural glucose metabolism that leads to an increased activity of the polyol pathway with accumulation of sorbitol

and fructose and a subsequent depletion of myo-inositol. Presumably one of the consequences of a myo-inositol depletion is a reduction of the Na,K ATPase activity and thus an impairment of nerve function (Finegold *et al.*, 1983; Dyck *et al.*, 1987). The other major hypothesis points to the reduction of endoneurial bloodflow and oxygen tension in nerves which have been extensively described in diabetic rats (Tuck *et al.*, 1984; Yasuda & Dyck, 1987). Rats subjected to chronic hypoxia (10%) developed electrophysiological abnormalities that were similar to those of EDN but in the absence of a dysregulation of the glucose level (Low *et al.*, 1984; 1986; 1987; Hendriksen *et al.*, 1992).

Different putative pharmacotherapies have been tested over the years that were aimed to prevent or improve diabetic neuropathy. One line of research focused on the efficacy of compounds that would interfere with the presumed aetiology of diabetic neuropathy e.g. regulating blood glucose levels by treatment with insulin (Greene *et al.*, 1975; Finegold *et al.*, 1983) or preventing flux through the polyol pathway by treatment with aldose reductase inhibitors (Mayer & Tomlinson, 1983; Tomlinson *et al.*, 1984; 1985). Another experimental approach tries to capitalize on the possibility that putative neurotrophic drugs may possess neuroprotective properties as well. In this respect it was demonstrated that the neurotrophic peptide, Org 2766 (a synthetic analogue of ACTH₄₋₉) known to facilitate recovery of function following a mechanical lesion of the sciatic nerve in the rat (Koning de & Gispen, 1987) also prevents the development of peripheral neuropathy induced by cisplatin (Hamers *et al.*, 1990) or by streptozotocin (EDN) (Van der Zee *et al.*, 1989).

The use of the Ca^{2+} antagonist, nimodipine, in the treatment of peripheral neuropathies is based on a similar rationale. In general, Ca^{2+} antagonists potentially modulate numerous aspects of nervous system function (Bär *et al.*, 1990).

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Following the discovery of specific binding sites for Ca^{2+} antagonists of the 1,4-dihydropyridine type in neural tissue (Bellemann *et al.*, 1983) it became clear that these dihydropyridines exert profound neuropharmacological effects (Traber & Gispen, 1989). Special attention was paid to nimodipine (isopropyl(2-methoxy-ethyl) 1,4-dihydro 2,6 dimethyl-4(3-nitrophenyl)-3,5-pyridine dicarboxylate) as this compound readily penetrates the blood brain barrier (Hoffmeister *et al.*, 1985). The neurotrophic and neuroprotective action of nimodipine was investigated, under a variety of experimental conditions: neurones in culture (Azmitia, 1989), post-lesion recovery of function in PNS and CNS (Van der Zee *et al.*, 1987; 1991; Nyakas *et al.*, 1989; Gispen *et al.*, 1991) age-related brain and behavioural deficits in rodents (Schuurman *et al.*, 1987; Disterhoft *et al.*, 1989; Landfeld, 1989) and cisplatin-induced, peripheral neuropathy in rats (Hamers *et al.*, 1990). Furthermore as a member of the dihydropyridine class of Ca^{2+} channels blockers, nimodipine may exhibit vasodilating effects that may have a beneficial effect on endoneurial bloodflow (Brandt *et al.*, 1981). In view of the interesting combination of pharmacological effects as discussed above, it was decided to test the putative efficacy of nimodipine in EDN. Recently we demonstrated that treatment of STZ-induced diabetic rats with nimodipine 20 mg kg^{-1} i.p. from the start of the diabetes counteracts the development of EDN (Kappelle *et al.*, 1992). In view of these preventive properties of nimodipine in EDN, it was of interest to investigate the possibility that nimodipine might also ameliorate an existing EDN. We therefore studied the effect of nimodipine on existing neuropathy in young and adult STZ-induced, diabetic rats.

Methods

Animals

Male Wistar rats of an inbred strain (TNO, Zeist, NL) weighing approximately 300 g at the start of the first two experiments (age 13–14 weeks) were used. In a third experiment, rats weighing approximately 570 g (age 12 months) were used. 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*. The rats were made diabetic with a single intravenous injection of streptozotocin (Zanosar, Upjohn Company, Kalamazoo, Michigan, U.S.A.) at a dose of 40 mg kg^{-1} body weight in experiments 1 and 2. In experiment 3 the rats received a single intravenous injection of STZ at a dosage of 20 mg kg^{-1} . Samples for blood glucose monitoring were obtained from the tail vein. After 48 h blood glucose levels were determined with a Minilab (Bayer GmbH, Munich, Germany). Rats with glucose levels $>15 \text{ mmol l}^{-1}$ 48 h following STZ injection were considered diabetic. Age-matched, non-diabetic rats served as controls.

Drugs

Nimodipine (Bayer AG, Elberfeld, Germany) as a micronized powder was dissolved in polyethylene glycol 1 ml kg^{-1} under dim light and injected intraperitoneally as a freshly prepared solution. Control rats received 1 ml kg^{-1} polyethylene glycol 400 i.p. In all 3 experiments nimodipine or placebo (polyethylene glycol) treatment began once a peripheral neuropathy had developed i.e. 4 weeks following STZ treatment.

Electrophysiology

Measurements of nerve conduction velocities were carried out under general anaesthesia (Hypnorm, Duphar, Weesp, NL, containing fluanisone 10 mg ml^{-1} and fentanyl citrate 0.2 mg ml^{-1} , dose 0.8 ml kg^{-1} body weight, administered subcutaneously). To minimize effects of differences in body

temperature on conduction velocity, the animals were placed under a 40-W light bulb (at a distance of 25 cm) 5 min prior to testing. During this procedure, the rectal temperature varied from $37\text{--}38^\circ\text{C}$. The method is described in detail by De Koning & Gispen (1987). In short, the sciatic and tibial nerves were stimulated at the sciatic notch and ankle, respectively, by means of monopolar needle electrodes. The anode was placed 5 mm proximal to the cathode. Upon stimulation of these mixed peripheral nerves, two responses were recorded from the small muscles of the foot by means of surface electrodes: the short-latency M-response, due to stimulation of α -motor fibres, and the long-latency H-response, due to stimulation of afferent Ia-fibres, which excite α -motor neurones in the spinal cord monosynaptically (Waxman, 1980; Stanley, 1981). The motor nerve conduction velocity (MNCV) and H-reflex related sensory nerve conduction velocity (SNCV) can be calculated from the latencies of these responses and the distance between the two stimulation points. The measurements were performed in a 'blind' fashion to ensure that the experimenter, who measured the NCVs, was unaware of the treatment a rat had received.

Outline of the study

In all experiments one group served as an age-matched, control group (Con) and one group served as a diabetic control group (Plac), both treated with 1 ml kg^{-1} polyethylene glycol i.p. every 48 h. In the first and the third experiment a third, diabetic, group (Nimo 20) was treated with nimodipine 20 mg kg^{-1} i.p. every 48 h. In the second experiment one diabetic group (Nimo 5) was treated with nimodipine 5 mg kg^{-1} i.p. every 48 h and another diabetic group (Nimo 10) was treated with nimodipine 10 mg kg^{-1} i.p. every 48 h. In all experiments the nimodipine or placebo (polyethylene glycol) treatment was initiated 4 weeks after the STZ injection when EDN was manifest and continued for 6 weeks. SNCV and MNCV were measured at week 0, 4, 6, 8 and 10.

Data analysis

The treatment code was broken at the end of the experiment to allow analysis of the data obtained for the various treatment groups. All data are presented as means $\pm$ s.e.mean. The results of SNCV and MNCV and body weights were analysed by a multivariate analysis of variance with repeated measurements (MANOVA) followed by supplemental *t* tests.

Results

Body weight and blood glucose levels

The STZ-induced diabetic rats developed high blood glucose levels which increased within 48 h. The blood glucose levels remained $>15 \text{ mmol l}^{-1}$ throughout the experimental period of 10 weeks. Additional treatment with nimodipine did not influence the blood glucose levels in any experiment (Table 1).

There was a marked reduction in body weight after the induction of diabetes (ten weeks after STZ administration a mean loss of $36.7 \pm 7.7 \text{ g}$ in experiment 1, of $41.7 \pm 6.1 \text{ g}$ in experiment 2 and of $176 \pm 10.1 \text{ g}$ in experiment 3). The mean weight of the nimodipine and the polyethylene glycol-treated diabetic rats in all experiments were not significantly different during the studies (data not shown).

Effect of streptozotocin-induced diabetes on nerve conduction velocity

Age-matched controls showed an increase of MNCV and SNCV in experiments 1 and 2 while they showed hardly any change in the fully grown rats in experiment 3. In all 3 experiments the differences in both MNCV and SNCV

Table 1 Blood glucose level ($\text{mmol l}^{-1} \pm \text{s.e.mean}$) in diabetic rats

		Week 0	Week 4	Week 10
Exp 1:	Con	5.8 $\pm$ 0.5	6.4 $\pm$ 0.8	7.4 $\pm$ 0.7
	Plac	28.4 $\pm$ 1.4	28.5 $\pm$ 1.3	31.6 $\pm$ 1.8
	Nimo 20	27.0 $\pm$ 1.5	26.7 $\pm$ 0.8	29.4 $\pm$ 1.7
Exp 2:	Con	6.2 $\pm$ 0.7	6.7 $\pm$ 0.6	7.0 $\pm$ 0.5
	Plac	22.0 $\pm$ 0.4	25.2 $\pm$ 1.0	28.6 $\pm$ 1.1
	Nimo 5	25.1 $\pm$ 1.5	26.8 $\pm$ 1.4	29.2 $\pm$ 1.5
Exp 3:	Nimo 10	23.4 $\pm$ 0.8	27.1 $\pm$ 1.1	29.2 $\pm$ 1.5
	Con	7.8 $\pm$ 0.5	5.9 $\pm$ 0.9	7.2 $\pm$ 0.9
	Plac	28.1 $\pm$ 2.4	27.5 $\pm$ 2.0	29.6 $\pm$ 1.7
	Nimo 20	27.6 $\pm$ 1.3	27.7 $\pm$ 1.8	29.0 $\pm$ 2.1

The mean glucose levels $\pm$ s.e.mean 48 h after streptozotocin injection (Week 0), at the start of the treatment (Week 4) and at the end of the 3 experiments (Week 10) of the age-matched control groups (Con), the placebo-treated groups (Plac) and the nimodipine 5 mg kg^{-1} , 10 mg kg^{-1} and 20 mg kg^{-1} -treated groups (Nimo 5, Nimo 10, Nimo 20).

between age-matched controls and diabetic rats reached significance within 4 weeks (ANOVA followed by supplemental *t* test; experiment 1 and 2: SNCV and MNCV $P < 0.001$; experiment 3: SNCV, $P < 0.005$ and MNCV, $P < 0.001$). Diabetic rats treated with vehicle displayed hardly any change in MNCV and SNCV after STZ administration in experiment 1 and 2 but a marked decrease in experiment 3 (Figures 1–3).

Effect of nimodipine treatment on nerve conduction velocity

In all experiments, nimodipine treatment was started 4 weeks after STZ administration. In experiment 1, treatment with nimodipine, 20 mg kg^{-1} ($n = 12$) resulted in an increase in SNCV and MNCV as compared to the placebo treatment ($n = 12$) (SNCV: $F(1,22) = 14.37$, $P < 0.001$; MNCV: $F(1,22) = 13.79$, $P < 0.005$) (Figure 1).

In order to obtain more information on the dose-response relationship, two lower doses were tested. Nimodipine 10 mg kg^{-1} treatment ($n = 12$) also resulted in an increase of SNCV and MNCV as compared to the placebo treatment (SNCV: $F(1,20) = 8.24$, $P < 0.01$; MNCV: $F(1,20) = 7.76$, $P < 0.05$) but the gain in velocity was smaller than that observed following treatment with nimodipine 20 mg kg^{-1} . Nimodipine 5 mg kg^{-1} had no beneficial effect on SNCV or MNCV (SNCV: $F(1,21) = 0.00$, $P = 0.985$; MNCV: $F(1,21) = 0.00$, $P = 0.953$) (Figure 2).

In the last experiment we tested whether the beneficial effect of nimodipine was also present in fully mature, adult rats. Placebo-treated, one-year-old rats showed a severe decrease of SNCV and MNCV whereas treatment with nimodipine 20 mg kg^{-1} resulted in a slight increase of SNCV and MNCV (SNCV: $F(1,17) = 11.86$, $P < 0.005$; MNCV: $F(1,17) = 8.74$, $P < 0.01$) (Figure 3).

Discussion

The STZ-induced diabetic rat is used in several laboratories as a possible model for diabetic neuropathy. STZ causes chronic hyperglycaemia and a severe reduction in peripheral NCVs of the diabetic rats (Mordes & Rossini, 1981). The model allows the study of the efficacy of agents to counteract some of the effect of chronic hyperglycaemia on peripheral nerves. Based on the neuroprotective effects of nimodipine in the development of EDN in the STZ rat model (Kappelle *et al.*, 1992) it was decided to test the effect of nimodipine on an existing diabetic neuropathy. Such an effect could lead to a better understanding of the defects in diabetic rats and would

Figure 1 Sensory nerve conduction velocity (SNCV) (a) and motor nerve conduction velocity (MNCV) (b) $\pm$ s.e.mean (vertical bars) in diabetic rats. Treatment started at 4 weeks after streptozotocin (STZ) administration. Group 1 (Δ , $n = 12$), a non-diabetic, age-matched control group, in which rats were treated with placebo (polyethylene glycol). Group 2 (∇ , $n = 12$), in which diabetic rats were also treated with placebo i.p. every 48 h. Group 3 ($\circ$, $n = 12$) in which diabetic rats were treated with nimodipine 20 mg kg^{-1} i.p. every 48 h. Treatment with nimodipine (20 mg kg^{-1}), significantly enhanced the SNCV compared to that of the treatment of placebo ($F(1,22) = 14.37$, $P < 0.001$). Treatment with nimodipine (20 mg kg^{-1}) also significantly enhanced the MNCV as compared to that of the placebo-treated group ($F(1,22) = 13.79$, $P < 0.005$).

be helpful in the development of putative treatment strategies in patients with type 1 or type 2 diabetes mellitus with neuropathy.

Nimodipine treatment affected neither body weight nor blood glucose levels and therefore did not interfere with the diabetic state. It also has no effects on the NCV of young, non-diabetic, control rats (Hamers *et al.*, 1991). As both MNCV and SNCV of the non-diabetic, mature, control rats in experiment 3 did not significantly change throughout the treatment period it seems that polyethylene glycol did not influence NCVs. It is clear from the electrophysiological data that nimodipine ameliorates an existing diabetic neuropathy in a dose-dependent manner. The drug is effective in a dosage of 10 mg kg^{-1} and 20 mg kg^{-1} per 48 h whereas 5 mg kg^{-1} is not. It is of interest to note that the observed dose-response relationship is identical to that observed for the neurotrophic action of nimodipine on the recovery of function following a crush lesion of the sciatic nerve in rats (Gispén *et al.*, 1991). The increase of SNCV from week 4 (start of the treatment) until week 10 (end of the treatment) in the nimodipine 20 mg kg^{-1} -treated group was 4.2 m s^{-1} and 4.0 m s^{-1} for the MNCV, whereas the nimodipine 10 mg kg^{-1} -treated group showed an increase of 2.1 m s^{-1} for SNCV and 3.4 m s^{-1} for MNCV. The use of young adult rats has the disadvantage that these rats are still maturing and hence the NCVs are increasing the time to approximately 60 m s^{-1} (Kappelle *et al.*, 1992). Therefore drug effects under these experimental conditions might be related not only to EDN but also to

Figure 2 Sensory nerve conduction velocity (SNCV) (a) and motor nerve conduction velocity (MNCV) (b) $\pm$ s.e.mean in diabetic rats. Treatment started at 4 weeks after streptozotocin (STZ) administration. Group 1 ($\blacktriangle$, $n = 10$), a non-diabetic, age-matched control group, was treated with placebo (polyethylene glycol) i.p. every 48 h. Group 2 (∇ , $n = 12$), diabetic rats were also treated with placebo i.p. every 48 h. Group 3 (Δ , $n = 12$) diabetic rats which were treated with nimodipine 5 mg kg⁻¹ i.p. every 48 h. Group 4 ($\circ$, $n = 12$), diabetic rats which were treated with nimodipine 10 mg kg⁻¹ i.p. every 48 h. Treatment with nimodipine 10 mg kg⁻¹ significantly enhanced the SNCV as compared to that of the placebo-treated group ($F(1,20) = 8.24$, $P < 0.01$). Treatment with nimodipine 5 mg kg⁻¹ body weight did not significantly enhance the SNCV as compared to that of the placebo-treated group ($F(1,21) = 0.00$, $P = 0.985$). Treatment with nimodipine 10 mg kg⁻¹ also significantly enhanced the MNCV as compared to that of the placebo-treated group ($F(1,20) = 7.76$, $P < 0.05$). Treatment with nimodipine 5 mg kg⁻¹ did not significantly enhance the MNCV as compared to that of the placebo-treated group ($F(1,21) = 0.00$, $P = 0.953$).

maturation processes. Hence we investigated whether nimodipine could ameliorate EDN in STZ-induced, one-year-old diabetic rats that were fully matured. Indeed we demonstrated that nimodipine 20 mg kg⁻¹ not only ameliorates EDN in young adult rats but also in fully grown, one-year-old, adult rats.

Within the experimental period there was no complete restoration of NCVs by nimodipine as compared to the level of the non-diabetic, age-matched controls. This could be due to the fact that diabetic rats have high blood glucose levels, which results in a worsening of their physical condition. Since diabetic neuropathy is a multifactorial disease, nimodipine could ameliorate just one aspect of the disease, whereas other factors such as depletion of trophic factors also need to be corrected to find complete restoration.

The mechanism of the neurotrophic and neuroprotective

Figure 3 Sensory nerve conduction velocity (SNCV) (a) and motor nerve conduction velocity (MNCV) (b) $\pm$ s.e.mean in diabetic rats. Treatment started at 4 weeks after streptozotocin (STZ) administration. Group 1 ($\blacktriangle$, $n = 10$), a non-diabetic, age-matched control group, in which rats were treated with placebo (polyethylene glycol) i.p. every 48 h. Group 2 (∇ , $n = 10$), in which diabetic rats were also treated with placebo i.p. every 48 h. Group 3 (Δ , $n = 10$) diabetic rats which were treated with nimodipine 20 mg kg⁻¹ i.p. every 48 h. Treatment with nimodipine 20 mg kg⁻¹ significantly enhanced the SNCV as compared to that of the placebo-treated group ($F(1,17) = 11.86$, $P < 0.005$). Treatment with nimodipine 20 mg kg⁻¹ also significantly enhanced the MNCV as compared to that of the placebo-treated group ($F(1,17) = 8.74$, $P < 0.01$).

action of nimodipine is still unclear. There is increasing evidence suggesting a vascular implication in the pathogenesis of diabetic neuropathy. Reduced nerve blood flow and endoneurial hypoxia have been demonstrated (Low *et al.*, 1987). It is possible that nimodipine has a vasodilator effect on the microvascular vessels (Brandt *et al.*, 1981), improving endoneurial bloodflow and hence oxygen delivery to the peripheral nerves. A similar mechanism has been demonstrated using angiotensin converting enzyme (ACE) inhibition (Cameron *et al.*, 1991). Increased oxygen delivery could improve oxygen-dependent metabolic processes, leading to better nerve function. In this respect it is of interest to note that nimodipine treatment may affect the endoneurial blood flow of the sciatic nerve in the STZ-induced diabetic rat. Treatment started 4 weeks after the induction of diabetes and lasted for a period of 6 weeks. Nimodipine restored the deficit in endoneurial bloodflow by about 50% (Kappelle *et al.*, unpublished observations). Alternatively enhanced blood supply may also originate from the reported influence of nimodipine on microvessel growth (Yuan *et al.*, 1990).

Accumulating evidence suggests that Ca²⁺ plays an important role in cell injury. Changes in [Ca²⁺] might play a role in the pathogenesis of diabetic neuropathy since [Ca²⁺] is in-

creased in the axoplasm from myelinated fibres of diabetic rats (Lowery *et al.*, 1990). Changes in $[Ca^{2+}]$ could lead to a diminished action of the Na,K ATP-ase (Pan & Janus, 1984; Glossmann *et al.*, 1989). Na,K ATP-ase is an important enzyme for maintaining the exact balance between the intracellular potassium and extracellular sodium concentration, which is crucial in the propagation of action potentials in the peripheral nerves.

It should be noted that we and others have previously described neuroprotective effects in EDN of compounds that clearly have quite different mechanisms of action. The compounds studied include aldose reductase inhibitors (Mayer *et*

al., 1983), myo-inositol (Finegold *et al.*, 1983) and neurotrophic peptides (Van der Zee *et al.*, 1989; Bravenboer *et al.*, 1991). Apparently the complex nature of the pathogenesis of EDN allows improvement of nerve function through interference with different components of the disease process or with interference of the etiological mechanisms via different routes.

We conclude that nimodipine is not only effective in preventing the development of EDN (Kappelle *et al.*, 1992), but also effective in ameliorating existing EDN. Further studies are necessary to unravel the mechanisms by which nimodipine is capable of ameliorating an existing EDN.

References

- AZMITIA, E.C. (1989). Nimodipine attenuates NMDA- and MDMA-induced toxicity of cultured fetal serotonergic neurons: evidence for a generic model of calcium toxicity. In *Nimodipine and Central Nervous System Function: New Vistas*. ed. Traber, J. & Gispén, W.H. pp. 141–159. Stuttgart-New York: Schattauer.
- BÄR, P.R., TRABER, J., SCHUURMAN, T. & GISPEN, W.H. (1990). CNS and PNS effects of nimodipine. *J. Neural Transm.*, **31**, 55–71.
- BELLEMAN, R., SCHADE, A. & TOWART, R. (1983). Dihydropyridine receptor in rat brain labelled with (3H)Nimodipine. *Proc. Natl. Acad. Sci. U.S.A.*, **80**, 2356–2360.
- BRANDT, L., ANDERSSON, K.E., EDVINSSON, L. & LIUNGGREN, B. (1981). Effect of extracellular calcium antagonists on the contractile responses of isolated human pial and mesenteric arteries. *J. Cerebr. Blood Flow Metabol.*, **3**, 339–347.
- BRAVENBOER, B., KAPPELLE, A., BUREN VAN, T., ERKELENS, D.W. & GISPEN, W.H. (1991). ACTH-analogue Org 2766 can ameliorate existing neuropathy in diabetic rats. *Diabetologia*, **34**, 619.
- CAMERON, N.E., COTTER, M.A. & ROBERTSON, S. (1991). Angiotensin converting enzyme inhibition prevents development of muscle and nerve dysfunction and stimulates angiogenesis in streptozotocin-diabetic rats. *Diabetologia*, **35**, 12–18.
- CRISA, L., MORDES, J.P. & ROSSINI, A.A. (1992). Autoimmune diabetes mellitus in the BB rat. *Diabetes Met. Rev.*, **8**, 9–37.
- DISTERHOFT, J.F., DEYO, R.A., BLACK, J., JONGE DE, M., STRAUPE, K.T. & THOMPSON, L.T. (1989). Associative learning in aging rabbits is facilitated by nimodipine. In *Nimodipine and Central Nervous System Function: New Vistas*. ed. Traber, J. & Gispén, W.H. pp. 209–226. Stuttgart-New York: Schattauer.
- DYCK, P.J., JAMES, J. & O'BRIEN, P.C. (1987). Diagnosis staging and classification of diabetic neuropathy and association with other complications. In *Diabetic Neuropathy*, ed. Dyck, P.J., Thomas, P.K., Asbury, A.K., Winegrad, A.I. & Porte, D. pp. 36–44. Philadelphia: WB Saunders Co.
- FINEGOLD, D., LATTIMER, S.A., NOLLE, S., BERNSTEIN, M. & GREENE, D.A. (1983). Polyol pathway activity and myo-inositol metabolism: a suggested relationship in pathogenesis of diabetic neuropathy. *Diabetes*, **32**, 988–992.
- GISPEN, W.H., SPOREL-ÖZAKAT, E., DUCKERS, E. & EDWARDS, P.M. (1991). The Ca^{2+} antagonist nimodipine and the recovery of function following peripheral nerve damage in the rat. *Neurosci. Res. Commun.*, **8**, 175–184.
- GLOSSMANN, H., ZERNIG, G., GRAZIADEI, I. & MOSHAMMER, T. (1989). Non L-type Ca^{2+} channel linked receptors for 1,4-dihydropyridines and phenylalkylamines. In *Nimodipine and Central Nervous System Function: New Vistas*. ed. Traber, J. & Gispén, W.H. pp. 51–67. Stuttgart-New York: Schattauer.
- GREENE, D.A., LATTIMER, S.A. & SIMA, A.A.F. (1987). Sorbitol, phosphoinositides and sodium-potassium-ATP-ase in the pathogenesis of diabetic complications. *N. Engl. J. Med.*, **316**, 599–606.
- GREENE, D.A., DEJESUS, P.V. JR & WINEGRAD, A.I. (1975). Effects of insulin and dietary myoinositol on impaired peripheral motor nerve conduction velocity in acute streptozotocin diabetes. *J. Clin. Invest.*, **55**, 1326–1336.
- HAMERS, F.P.T., GERRITSEN VAN DER HOOP, R., TRABER, J., NEIJT, J.P., JENNEKENS, F.G.I. & GISPEN, W.H. (1990). The calcium antagonist nimodipine in cisplatin-induced neuropathy in the rat. *Eur. J. Pharmacol.*, **183**, 1710–1711.
- HAMMERS, F.P.T., GERRITSEN VAN DER HOOP, R., STEERENBURG, P.A., NEIJT, J.P. & GISPEN, W.H. (1991). Putative neurotrophic factors in the protection of cisplatin-induced peripheral neuropathy in rats. *Toxicol. Appl. Pharmacol.*, **111**, 514–522.
- HENDRIKSEN, P.H., OEY, P.L., WIENEKE, G.H., HUFFELEN VAN, A.C. & GISPEN, W.H. (1992). Hypoxic neuropathy versus diabetic neuropathy – an electrophysiological study in rats. *J. Neurol. Sci.*, **110**, 99–106.
- HOFFMEISTER, F., BELLEMANN, H.P., BENZ, U. & VAN DEN KERCKHOFF, W. (1985). Psychotropic actions of nimodipine. In *Nimodipine: Pharmacological and Clinical Properties*. ed. Betz, E., Deck, K. & Hoffmeister, F. pp. 77–97. Stuttgart-New York: Schattauer.
- KAPPELLE, A.C., BRAVENBOER, B., TRABER, J., ERKELENS, D.W. & GISPEN, W.H. (1992). The Ca^{2+} antagonist nimodipine counteracts the onset of an experimental diabetic neuropathy in streptozotocin-induced diabetic rats. *Neurosci. Res. Commun.*, **10**, 95–104.
- KONING DE, P. & GISPEN, W.H. (1987). Org 2766 improves functional and electrophysiological aspects of regenerating sciatic nerve in the rat. *Peptides*, **8**, 415–422.
- LANDFIELD, P.W. (1989). Nimodipine modulation of aging-related increases in hippocampal calcium currents. In *Nimodipine and Central Nervous System Function: New Vistas*. ed. Traber, J. & Gispén, W.H. pp. 227–238. Stuttgart-New York: Schattauer.
- LOW, P.A., SCHMELZER, J.D., WARD, K.K. & YAO, J.K. (1986). Experimental chronic hypoxic neuropathy; relevance to diabetic neuropathy. *Am. J. Physiol.*, **250**, E94–99.
- LOW, P.A., TUCK, R.R., DYCK, P.J., SCHMELZER, J.D. & YAO, J.K. (1984). Prevention of some electrophysiologic and biochemical abnormalities with oxygen supplementation in experimental diabetic neuropathy. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 6894–6898.
- LOW, P.A., TUCK, R.R. & TAKEUCHI, M. (1987). Nerve microenvironment in diabetic neuropathy. In *Diabetic Neuropathy*. ed. Dyck, P.J., Thomas, P.K., Asbury, A.K., Winegrad, A.I. & Porte, D. pp. 266–278. Philadelphia: Saunders.
- LOWERY, J.M., EICHBERG, J., SAUBERMANN, A.J. & LOPACHIN JR, M. (1990). Distribution of elements and water in peripheral nerve of streptozotocin-induced diabetic rats. *Diabetes*, **39**, 1498–1503.
- MAYER, J.H. & TOMLINSON, D.R. (1983). Prevention of defects of axonal transport and nerve conduction velocity by oral administration of myo-inositol or an aldose-reductase inhibitor in streptozotocin-diabetic rats. *Diabetologia*, **25**, 433–438.
- MORDES, J.P. & ROSSINI, A.A. (1981). Animal models of diabetes. *Am. Med.*, **70**, 353–360.
- NYAKAS, C., MARKEL, E., KRAMERS, R.J.K., GASPAR, E., BOHUS, B. & LUITEN, P.G.M. (1989). Effects of nimodipine on hypoxia-induced learning and memory deficits. In *Nimodipine and Central Nervous System Function: New Vistas*. ed. Traber, J. & Gispén, W.H. pp. 175–194. Stuttgart-New York: Schattauer.
- PAN, M. & JANUS, R.A. (1984). Stimulation of Na,K-ATPase of isolated smooth muscle membranes by the Ca^{2+} channel inhibitors, nimodipine and nitrendipine. *Biochem. Pharmacol.*, **33**, 787–793.
- PIRART, J. (1978). Diabetes mellitus and its degenerative complications: a prospective study of 4400 patients observed between 1947 and 1973. *Diabetes Care*, **1**, 168–252.
- RERUP, C.C. (1970). Drugs producing diabetes through damage of the insulin secreting cells. *Pharmacol. Rev.*, **22**, 485–518.
- SCHUURMAN, T., KLEIN, H., BENEKE, M. & TRABER, J. (1987). Nimodipine and motor deficits in the aged rat. *Neurosci. Res. Commun.*, **1**, 9–15.
- STANLEY, E.F. (1981). Sensory and motor nerve conduction velocities and the latency of the H-reflex during growth of the rat. *Exp. Neurol.*, **71**, 497–506.

- THOMAS, P.K. & ELIASSON, S.G. (1975). Diabetic neuropathy. In *Peripheral Neuropathy*. ed. Dyck, P.J., Thomas, P.K. & Lambert, E.M. pp. 956–981. Philadelphia: P.A. Saunders.
- TOMLINSON, D.R., MORIARTY, R.J. & MAYER, J.H. (1984). Prevention and reversal of defective axonal transport and motor nerve conduction velocity in rats with experimental diabetes by treatment with the aldose reductase inhibitor sorbinil. *Diabetes*, **33**, 470–476.
- TOMLINSON, D.R., TOWNSEND, J. & FRETTE, P. (1985). Prevention of defective axonal transport in STZ diabetic rats by treatment of Statil (ICI 128,436), an aldose reductase inhibitor. *Diabetes*, **34**, 907–912.
- TRABER, J. & GISPEN, W.H. (1989). *Nimodipine and the Central Nervous System*. New Vistas. Stuttgart-New York: Schattauer.
- TUCK, R.R., SCHMELZER, J.D. & LOW, P.A. (1984). Endoneurial blood flow and oxygen tension in sciatic nerves of rats with experimental diabetic neuropathy. *Brain*, **107**, 935–950.
- WAXMAN, S.G. (1980). Determination of conduction velocity in myelinated nerve fibres. *Muscle Nerve*, **3**, 141–150.
- YASUDA, H. & DYCK, P.J. (1987). Abnormalities of endoneurial microvessels and sural nerve pathology in diabetic neuropathy. *Neurology*, **37**, 20–28.
- YUAN, X.Q., SMITH, T.L., PROUGH, D.S., WITT DE, D.S., DUSSEAU, J.W., LYNCH, C.D., FULTON, J.M. & HUTCHINS, P.M. (1990). Long-term effects of nimodipine on pial microvasculature and systemic circulation in conscious rats. *Am. J. Physiol.*, **258**, H1395–H1401.
- ZEE VAN DER, C.E.E.M., BRAKKEE, J.H. & GISPEN, W.H. (1991). Putative neurotrophic factors and functional recovery from peripheral nerve damage in the rat. *Br. J. Pharmacol.*, **103**, 1041–1046.
- ZEE VAN DER, C.E.E.M., GERRITSEN VAN DER HOOP, R. & GISPEN, W.H. (1989). Beneficial effect of peptide ORG 2766 (ACTH_{4,9} analog) in the treatment of peripheral neuropathy in streptozocin-diabetic rats. *Diabetes*, **38**, 225–230.
- ZEE VAN DER, C.E.E.M., SCHUURMAN, T., TRABER, J. & GISPEN, W.H. (1987). Oral administration of nimodipine accelerates functional recovery following peripheral nerve damage in the rat. *Neurosci. Lett.*, **83**, 143–148.

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