Protective effect of stobadine on NCV in streptozotocin-diabetic rats: augmentation by vitamin E

S. Skalska[1], Z. Kyselova[1], A. Gajdosikova[1], C. Karasu[2], M. Stefek[1] and S. Stolc[1]

[1] *Institute of Experimental Pharmacology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia*
[2] *Department of Medical Pharmacology, Faculty of Medicine, Gazi University, Ankara, Turkey*

Abstract. Hyperglycaemia-induced oxidative stress makes an important contribution to the aetiology of diabetic neuropathy. Elevated reactive oxygen species (ROS) cause cumulative damage to neurons and Schwan cells, however, they also have a deleterious effect on nerve blood flow causing endoneurial hypoxia, which is responsible for early nerve conduction velocity (NCV) deficits and contributes to an increase in resistance to ischaemic conduction failure (RICF).

We tested whether antioxidants – stobadine, vitamin E or the combination of these drugs, could prevent the early signs of neural dysfunction in animal model of diabetes in 8–9 weeks old male Wistar rats, made diabetic by streptozotocin (55 mg/kg i.v.) 4 months prior to testing. Neuropathy was evaluated electrophysiologically by measuring motor NCV and RICF of sciatic nerve *in vitro*. We observed that treatment with the combination of stobadine and vitamin E significantly ($p < 0.001$) reduced the NCV slowing in diabetic rats, although it did not fully prevent the NCV impairment. Significant effect ($p < 0.05$) was observed also in stobadine monotherapy. The RICF elevated in diabetic animals was not affected by any drug applied. This study confirmed that treatment with appropriate antioxidants, especially their combination could partially prevented the decrease in NCV in diabetic rats.

Key words: Diabetes — Neuropathy — Oxidative stress — Stobadine — Rats

Introduction

Diabetic neuropathy occurs through diverse pathogenic mechanisms, most of them being initiated by hyperglycaemia and oxidative stress (Baynes 1991; van Dam et al. 1995; Pop-Busui et al. 2006).

Several factors promote oxidative stress in diabetes, including increased free radical production caused by autoxidation reactions of sugars with proteins and unsaturated lipids (Baynes 1991; Sima and Sugimoto 1999), nerve ischaemia-reperfusion (Schmeltzer et al. 1989; Wang et al. 2004) and impairment of tissue anti-oxidant protection systems (Low and Nickander 1991).

Thus it is likely that reactive oxygen species (ROS) affect neurons and Schwann cells directly as well as indirectly *via* vascular effects. ROS have effects on blood vessel function, which compromise perfusion of several organs including peripheral nerves (Cameron and Cotter 1999; Camera et al. 2008).

Recent data stress the importance of mutual interactions between metabolic mechanisms at the endothelial level resulting in perfusion abnormalities (Cameron and Cotter 1995; Cameron et al. 1997; Nangle et al. 2006).

Early impairment of endoneurial nutritive flow leads to insufficient perfusion and nerve ischaemia in diabetic state (Wang et al. 2004). The subsequent acute impairment in nerve conduction velocity (NCV) and the enhanced resistance to ischaemic conduction failure (RICF) (Cameron et al. 1993; Bíró et al. 1997; Cameron and Cotter 1999) parallels a progressive defect in the paranodal barrier system (Sima et al. 1993). This defect is a consequence of oxidative modification of structural proteins and lipids (Schmeltzer et al. 1989; Baynes 1991; Sima et al. 1993). Endothelial cell dysfunction might underlie the focally-increased vascular permeability observed in diabetic neuropathy (Eaton et al. 1996).

Correspondence to: Silvia Skalska, Institute of Experimental Pharmacology, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia
E-mail: silvia.skalska@yahoo.com

Increased glucose, fructose, and glycogen stores, and perhaps reduced energy requirements in diabetic nerves explains in large part RICF (Low et al. 1985; Sango et al. 1995), an adaptive mechanism that increases the ability of nerve to cope with insufficient perfusion.

Some antioxidants, such as vitamin E, β-carotene, butylated hydroxytoulene, probucol and vitamin C, can improve the hypoperfusion of vasa nervorum and the ischaemic nerve abnormalities in diabetic rats, as assessed by NCV and RICF (van Dam and Bravenboer 1997; Cameron and Cotter 1999; Nangle et al. 2006).

Stobadine (Horakova and Stolc 1998) has antioxidant properties and the ability to scavenge ROS, such as hydroxyl, peroxyl, and alkoxyl radicals. Also, it is an effective quencher of singlet oxygen. Against superoxide radical, however, it exhibits only a low scavenging effect. Under conditions of experimental glycation model *in vitro*, stobadine was found to protect bovine serum albumin against glycooxidative damage (Stefek et al. 1996). These findings, along with the high oral bioavailability of stobadine, its toxic safety as well as efficient detoxification pathways, render this drug a prospective agent in the prevention of late diabetic complications (Stefek et al. 2000, 2002; Sotnikova et al. 2001, 2006; Ulusu et al. 2003).

More than one type of ROS is important for diabetic neural and neurovascular deficits to develop (Cotter et al. 1995; Nangle et al. 2006). The probability to achieve the desired concentration might be enhanced by simultaneous administration of several appropriately selected antioxidants affecting different targets. The antioxidant potency of stobadine may be increased by its interaction with other antioxidants with more negative redox-potentials (e.g. vitamin E), which might support its antioxidant action in physiological systems (Kagan et al. 1993).

The aim of the present study was to investigate the effect of stobadine, vitamin E, and combined stobadine and vitamin E dietary supplementation on NCV and RICF in 8–9 weeks old male Wistar rats with streptozotocin-induced hyperglycaemia. The well-established model of diabetes mellitus type I (van Dam et al. 1999; Terata et al. 1999; Coppey el al. 2001) was used. The overall physical and metabolic state of rats was also assessed in the course of the experiment.

Materials and Methods

Experimental diabetes

The study was approved by the Ethics Committee of the Institute and performed in accordance with the Principles of Laboratory Animal Care (NIH publication 83-25, revised 1985) and the Slovak law regulating animal experiments (Decree 289, Part 139, July 9th 2003). Male Wistar rats, 8–9 weeks old, weighing 230–250 g, were used. The laboratory animals were of monitored conventional quality and were supplied by the Breeding Facility of the Institute of Experimental Pharmacology at Dobrá Voda (Slovakia). Experimental diabetes was induced by a single *i.v.* dose of streptozotocin (55 mg/kg). Streptozotocin was dissolved in 0.1 mol/l citrate buffer, pH 4.5. The laboratory animals were fasted overnight prior to streptozotocin administration. Induction of diabetes with streptozotocin was done according to Larsen et al. (2002) modified and applied to rats. After streptozotocin injection, the rats had free access to glucose solution (5 mmol/l) to avoid and/or attenuate subsequent inevitable hyperinsulinemia and hypoglycaemic shock. No animals perished along this procedure. Control animals received 0.1 mol/l citrate buffer. Ten days after streptozotocin administration, all animals with fasting plasma glucose level >15 mmol/l were considered diabetic and were included in the study. During the experiment, the animals were housed in groups of two in cages of the type T4 Velaz (Prague, Czech Republic) with wood shaving bedding exchanged daily. Tap water and pelleted standard diet KKZ-P-M (Dobrá Voda, Slovakia) was available ad libitum. The animal room was air-conditioned and the environment was continuously monitored for the temperature of 23 ± 1°C and relative humidity of 40–70%. The animals were kept under a stable regimen of 12 h light/12 h darkness.

Experimental groups

Control and diabetic animals were randomly assigned to following groups comprising 4 up to 8 animals:
C – control rats kept on standard diet,
STB – control rats kept on standard diet supplemented with stobadine dipalmitate,
Vit.E – control rats treated with vitamin E,
Vit.E+STB – control rats treated with the combination of stobadine and vitamin E,
STZ – streptozotocin-induced diabetic rats on standard diet,
STZ+STB – streptozotocin-induced diabetic rats fed by diet supplemented with stobadine,
Vit.E+STZ – streptozotocin-induced diabetic rats treated with vitamin E,
STZ+Vit.E+STB – streptozotocin-induced diabetic rats treated with the combination vitamin E and stobadine.

The exact number of animals in a particular group is indicated in the paper separately. No animals died unwillingly prior to the regular end of the experiment.

The drugs were added to standard diet (KKZ-P-M) prior to its pelletization. The final concentration of stobadine and vitamin E in pellets was 0.05 and 0.1% (w/w), respectively. The content of the drugs was proved analytically. Animals had free access to food and drinking water. The above indicated concentration of the drugs in the diet was adjusted to reach the intended effective doses in diabetic animals, i.e. 21 mg of stobadine base/kg b.w. and 150 mg of vitamin E/kg b.w.

Blood measurement

Glycaemia and body weight were repeatedly followed during the experiment. Plasma glucose level was measured using a commercial glucose (Trinder) kit (Sigma, St. Louis, USA). After termination of the study (4 months after streptozotocin administration), NCV and RICF were measured in sciatic nerves *in vitro*.

NCV and RICF

Electrophysiological parameters of sciatic nerves, namely NCV and RICF were measured by methods essentially the same as those described by Cameron et al. (1991). Briefly, rats fasting overnight with free access to water were anaesthetized with thiopental (65 mg/kg i.p.). Both sciatic nerves were removed with branches supplying the gastrocnemius muscle. The nerves were stored in oxygenated (100% O_2) Krebs solution (pH = 7.3) consisting of (in mmol/l) NaCl 136; KCl 5.6; $CaCl_2 \cdot 2H_2O$ 2.2; $MgCl_2 \cdot 6H_2O$ 1.2; HEPES 5; glucose 4.9 until transferred to the experimental chamber. The chamber was filled with mineral oil pre-gassed with O_2 (100%). It comprised a system of platinum wire electrodes for stimulation, grounding and registration. Action potential was evoked by supramaximal (by 50%) square wave voltage pulses lasting 0.07 ms at the frequency 0.3 Hz. Compound action potential amplitude was monitored during adaptation period lasting typically 15 min. NCV was calculated in axons dominating in the preparation from the delay between stimulation artefact and the peak of the action potential amplitude, and the distance between the stimulation and recording electrodes. NCV was typically ~30 m/s, thus the axons belonged apparently to the A α group. After assessment of NCV, the O_2-equilibrated mineral oil was replaced by oil equilibrated with N_2 (100%) to asses RICF. The outcoming decline of the action potential was measured at 5-min intervals for the next 30 min. Differences between particular groups were evaluated in minute 30. All the measurements were made at 36°C.

Drugs and reagents used

Vitamin E (Hydrovit E forte, PharmaGal Slovakia, containing α-tocopherol acetate 300 mg/ml), stobadine dipalmitate (Institute of Experimental Pharmacology, SAS, Bratislava),

Table 1. Blood glucose levels (mmol/l) of rats in the 18 week experiment

Control groups (n = 4)			Diabetic groups (n = 8)		
Treatment	Day 10	Week 18	Treatment	Day 10	Week 18
C	5.2 ± 0.2	7.4 ± 0.3	STZ	16.1 ± 0.7	22.5 ± 0.8
STB	6.0 ± 0.1	5.8 ± 0.2	STZ+STB	15.9 ± 0.5	23.1 ± 1.6
Vit.E	4.6 ± 0.3	7.6 ± 0.5	STZ+Vit.E	15.9 ± 0.4	21.0 ± 1.1
STB+Vit.E	5.4 ± 0.2	6.2 ± 0.4	STZ+STB+Vit.E	16.7 ± 0.6	24.7 ± 0.9

C, control rats kept on standard diet; STB, control rats kept on standard diet supplemented with stobadine; Vit.E, control rats kept on vitamin E-supplemented food; STB+Vit.E, control rats treated with the combination of stobadine and vitamin E; STZ, streptozotocin-induced diabetic rats kept on standard diet;. STZ+STB, streptozotocin-induced diabetic rats kept on stobadine-supplemented food; STZ+Vit.E, streptozotocin-induced diabetic rats kept on vitamin E-supplemented food; STZ+STB+Vit.E, streptozotocin-induced diabetic rats kept on vitamin E and stobadine-supplemented food. Values shown are means ± SEM.

Table 2. Body weight (g) of rats in the 18 week experiment

Control groups (n = 4)			Diabetic groups (n = 8)		
Treatment	Day 10	Week 18	Treatment	Day 10	Week 18
C	239 ± 0.7	480 ± 11.2	STZ	242 ± 0.5	237 ± 4.9
STB	240 ± 0.6	438 ± 5.8	STZ+STB	240 ± 0.4	205 ± 7.1
Vit.E	232 ± 0.7	485 ± 11.4	STZ+Vit.E	238 ± 0.3	214 ± 7.4
STB+Vit.E	213 ± 0.4	464 ± 10.5	STZ+STB+Vit.E	231 ± 0.4	215 ± 1.4

C, control rats kept on standard diet; STB, control rats kept on standard diet supplemented with stobadine; Vit.E, control rats kept on vitamin E-supplemented food; STB+Vit.E, control rats treated with the combination of stobadine and vitamin E; STZ, streptozotocin-induced diabetic rats kept on standard diet;. STZ+STB, streptozotocin-induced diabetic rats kept on stobadine-supplemented food; STZ+Vit.E, streptozotocin-induced diabetic rats kept on vitamin E-supplemented food; STZ+STB+Vit.E, streptozotocin-induced diabetic rats kept on vitamin E and stobadine-supplemented food. Values shown are means ± SEM.

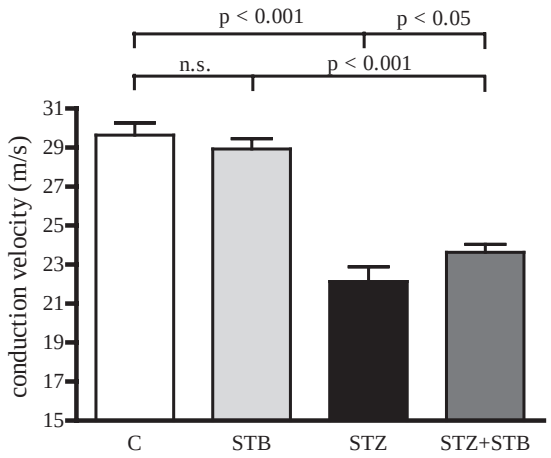

Figure 1. Conduction velocity in motor axons in rat sciatic nerves measured *in vitro* after 4 months of streptozotocin-induced diabetes. C, nerves (n = 14) from control rats kept on standard diet; STB, nerves (n = 7) from control rats kept on stobadine-supplemented food; STZ, nerves (n = 11) from streptozotocin-induced diabetic rats kept on standard diet; STZ+STB, nerves (n = 14) from streptozotocin-induced diabetic rats kept on stobadine-supplemented food; n.s., non significant. Means with SEM and significances are shown.

streptozotocin (Sigma), HEPES (Calbiochem), thiopental (Research Institute of Antibiotics and Biotransformations, Prague, Czech Republic), mineral oil (paraffinum liquidum, Czechoslovak Pharmacopoeia IV (SLAVUS, Bratislava, Slovakia). All other chemicals used were of analytical purity.

Statistical analysis

One way analysis of variance was performed and any significant ($p < 0.05$) differences were assigned to individual between-group comparisons using Student's t-test (GraphPad Instat, San Diego, CA, USA). All data are expressed as means ± SEM.

Results

Calculations of actual drug doses were based on average food consumption. In diabetic animals given stobadine and vitamin E, the average daily oral doses were 23.8 ± 0.3 mg/kg b.w. (calculated as base) and 168 ± 2 mg/kg b.w., respectively. The first measuring of blood glucose levels was made 10 days after induction of diabetes. The rats with hyperglycaemia ≥15 mmol/l were chosen for further study. Persistent hyperglycaemia, on average over 15 mmol/l, was recorded in diabetic animals throughout the whole experiment. The blood glucose levels in control rats were within the physiological range. Administration of any of the drugs had no effect on blood glucose levels. The average blood glucose levels of the control and diabetic experimental groups at

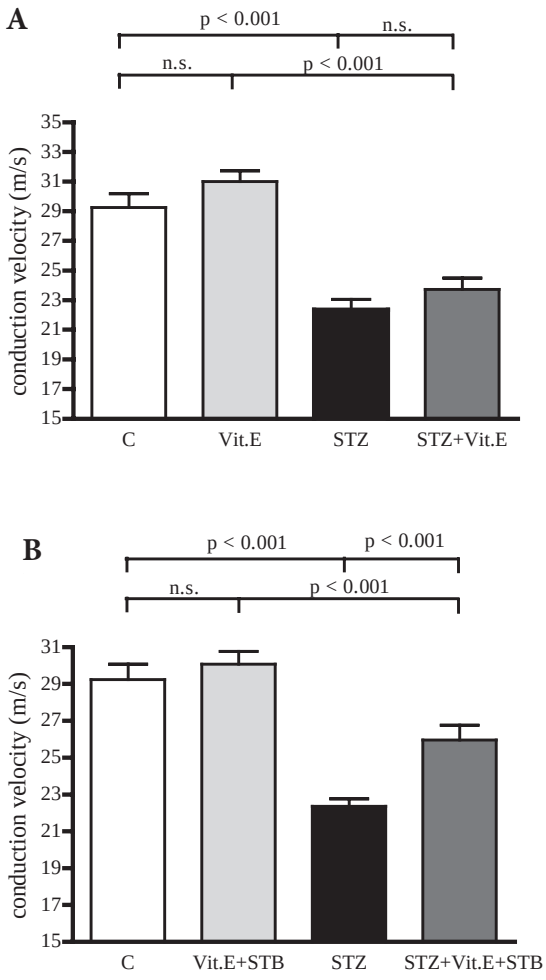

Figure 2. Action of vitamin E treatment (A) and treatment with combination of vitamin E and stobadine (B) on conduction velocity in motor axons in rat sciatic nerves measured *in vitro* after 4 months of streptozotocin-induced diabetes. Means with SEM are shown. C, nerves (n = 7) from control rats on standard diet; Vit.E, nerves (n = 8) from control rats kept on vitamin E-supplemented food; STZ, nerves (n = 14) from diabetic rats kept on standard diet; STZ+Vit.E, nerves (n = 15) from streptozotocin-induced diabetic rats kept on vitamin E monotherapy; Vit.E+STB, nerves (n = 7) from control rats on vitamin E and stobadine-supplemented food; STZ+Vit.E+STB, nerves (n = 13) from streptozotocin-induced diabetic rats on vitamin E and stobadine therapy.

the beginning and at the end of the 18-week experiment are given in Table 1.

Besides hyperglycaemia, the streptozotocin-treated animals revealed further typical signs of diabetes, such as polyphagia, polydipsia, and polyuria. Despite polyphagia, at the end of the experiment, the weights of diabetic rats were significantly lower as compared with those in the control group (Table 2). Non-diabetic rats were continuously gaining weight. Administration of the antioxidants did not significantly affect

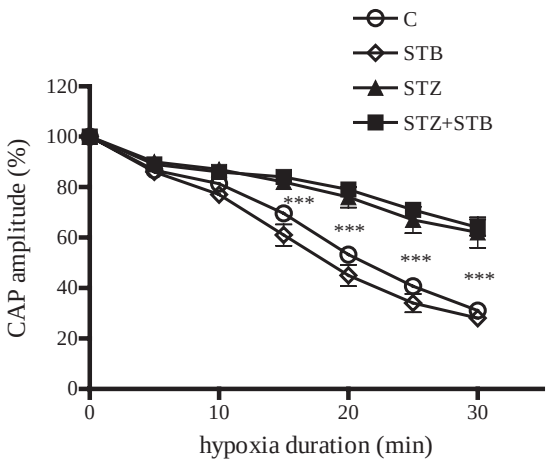

Figure 3. Change in sciatic nerve compound action potential (CAP) amplitude during hypoxia after 4 months of streptozotocin-induced diabetes. C, nerves (n = 14) from control rats kept on standard diet; STB, nerves (n = 7) from control rats kept on stobadine-supplemented food; STZ, nerves (n = 11) from streptozotocin-induced diabetic rats kept on standard diet; STZ+STB, nerves (n = 14) from streptozotocin-induced diabetic rats kept on stobadine-supplemented food. Means with SEM are shown. Differences in the resistance of nerve conduction to hypoxia between the diabetic and non-diabetic groups are significant (*** $p < 0.001$) since minute 15.

blood glucose and body weights either in control or diabetic animals.

After 4-month duration of diabetes, NCV in sciatic nerves from diabetic rats measured *in vitro* was significantly lower ($p < 0.001$) compared to that in nerves from control rats (Fig. 1). Stobadine treatment slightly mitigated this deteriorating effect of diabetes ($p < 0.05$), yet was far from preventing it. Therapy with vitamin E had no significant effect on this reduction (Fig. 2A). However, therapy with the combination of stobadine and vitamin E attenuated significantly ($p < 0.001$) this impairment compared to the untreated nerves (Fig. 2B), although it was not able to prevent the conduction impairment completely. Administration of stobadine, vitamin E or stobadine with vitamin E to control rats was without any significant effect on NCV.

During nerve exposure to hypoxia *in vitro*, the compound action potential amplitude was readily declining with increasing hypoxia duration (Fig. 3). However, the nerves from diabetic animals were less sensitive to hypoxia than the non-diabetic ones, with significance since minute 15 of hypoxia ($p < 0.001$). The treatment with stobadine in the dosage regimen used had no effect on the decline of compound action potential amplitude. RICF of diabetic nerves was not mitigated by either vitamin E monotherapy or by therapy with the combination of both antioxidants (Fig. 4).

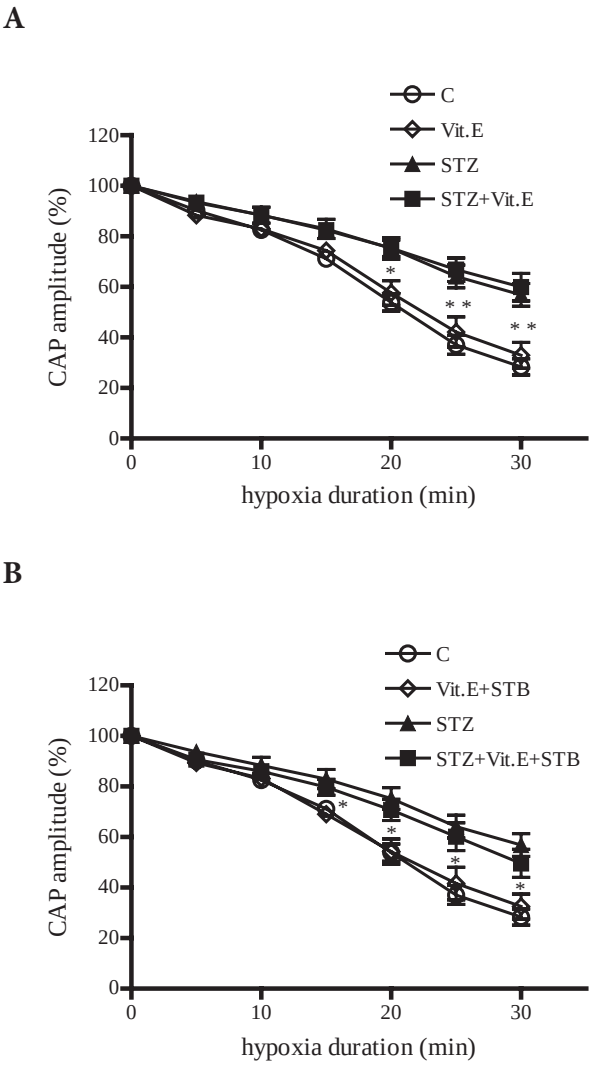

Figure 4. Action of vitamin E treatment (A) and treatment with combination of vitamin E and stobadine (B) on changes in sciatic nerve compound action potential (CAP) amplitude during hypoxia after 4 months of streptozotocin-induced diabetes. Means with SEM are shown, significances * $p < 0.05$, ** $p < 0.01$. Panel A: C, nerves (n = 7) from control rats kept on standard diet; Vit.E, nerves (n = 8) from control rats kept on vitamin E-supplemented food; STZ, nerves (n = 14) from streptozotocin-induced diabetic rats kept on standard diet; STZ+Vit.E, nerves (n = 15) from streptozotocin-induced diabetic rats kept on vitamin E therapy. Differences between non-diabetic and diabetic groups are significant since minute 20 of hypoxia. Panel B: C, nerves (n = 7) from control rats kept on standard diet; Vit.E+STB, nerves (n = 7) from streptozotocin-induced control rats kept on vitamin E and stobadine-supplemented food; STZ, nerves (n = 14) from streptozotocin-induced diabetic rats kept on standard diet; STZ+Vit.E+STB, nerves (n = 13) from streptozotocin-induced diabetic rats kept on vitamin E and stobadine therapy. Differences between non-diabetic and diabetic groups are significant since minute 15 of hypoxia.

Discussion

The main goal of our study was to assess the outcome of long-term hyperglycaemia on basic neuronal characteristics in sciatic nerves of streptozotocin-induced diabetic rats and subsequently to investigate the effect of selected antioxidants.

We observed a reduction in NCV and an increase in RICF in rats after 4-month duration of streptozotocin-induced diabetes, such was in accordance with previous investigations of other authors (Cameron et al. 1993; Bíró et al. 1997; van Dam et al. 1999).

Previous studies showed that high doses of vitamin E could partially prevented nerve conduction deficits in hyperglycaemic rats. Positive effects of vitamin E high doses (500 and 1000 mg/kg b.w., p.o.) on nerve conduction in streptozotocin-diabetes have been observed previously in maturing (Love et al. 1997) as well as in adult rats (Cotter et al. 1995). On the contrary, standard vitamin E supplementation of rat food (70 mg/kg of food) did not prevent neural dysfunction in young streptozotocin-diabetic rats (van Dam et al. 1999). When treatment was started during weaning, long-term vitamin E supplementation (190 mg/kg b.w., *i.p.* 3 times/week) also failed to affect NCV, endoneurial reduced glutathione or conjugated dienes and hydroperoxides (Nickander et al. 1994). Similarly, the vitamin E supplementation used in our study (1 g/kg of food) resulting in vitamin E intake 168 ± 2 mg/kg b.w. had no significant effect on reduction of NCV in diabetic rats after 4 month duration of diabetes. The finding is in agreement with the suggestion that pharmacological rather than physiological doses of natural antioxidants might be necessary to protect neurovascular function in diabetic rats (Cotter et al. 1995). This may in part be due to the higher levels of hyperglycaemia in experimental diabetes, than in patients with relatively well-controlled diabetes (Cotter et al. 1995).

The laboratory animals used in the present study were 8–9 weeks old at beginning. The maturation of the peripheral nerves in the rats of this age is not completed yet, which causes lower NCV than in adult rats (Wright and Nukada 1995). Indeed, this was confirmed also in our separate measurements (Fig. 5). Untreated diabetes may result both in growth retardation as well as in impairment of axonal conductivity. Maturational retardation may at least in part account for the differences observed in NCVs between immature control and diabetic rats. These growth-related changes in peripheral nerve make physiological and pathological assessment in immature diabetic animals more difficult to interpret. Thus, there is controversy about what extent the abnormalities, observed in immature diabetic animals, were due to hyperglycaemia-induced maturational delays or due to diabetes *per se* (Wright and Nukada 1995). Recent experiments indicated that antioxidant treatment,

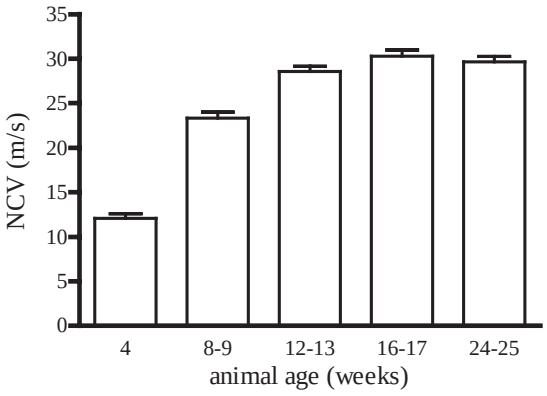

Figure 5. Increase in nerve conduction velocity (NCV) in motor axons in rat sciatic nerves during ontogenesis. Means with SEM are shown. NCV reached stable value 16–17 weeks after birth of the animal.

e.g. with very high doses of vitamin E, could protect against nerve dysfunction in experimental diabetes (Cotter et al. 1995; van Dam et al. 1999). However, protection may be somewhat less distinct in the maturing diabetic rats than in adult rats (van Dam et al. 1999).

The condition of the endotelium has considerable importance in optimal nerve function (Cameron and Cotter 1999; Camera et al. 2008). Consequently, oxidative stress may diminish endothelium-dependent vasodilatation. This was observed in chronic diabetic animals (Archibald et al. 1996) and even in resistance of isolated mesenteric artery in acute exposure to hyperglycaemia (Taylor and Poston 1994). Dietary supplementation of the antioxidant stobadine was proven to reduce vascular impairment in streptozotocin-diabetic rats (Sotnikova et al. 2001, 2006). Superoxide radicals react with NO radicals giving rise to peroxynitrite, an important source of hydroxyl radicals inducing endothelial damage (Beckman et al. 1990; Pieper et al. 1993). Stobadine could protect endothelial damage by scavenging hydroxyl radicals, although its effect against superoxide radical is not so evident (Horakova and Stolc 1998).

Stobadine was able to attenuate lipoxidation reactions in the diabetic heart, liver (Pekiner et al. 2002) and the kidneys (Stefek et al. 2000). We suggest, this ability of stobadine may account, at least partly, for its observed neuroprotective action, too.

The combination of stobadine and vitamin E significantly improved reduction of NCV, reaching a higher extent than monotherapy by stobadine. This is in agreement with the finding that antioxidant potency of stobadine may be increased by its interaction with other lipid- or water-soluble antioxidants with more negative redox-potentials (e.g. vitamins E and C) (Kagan et al. 1993). Stobadine has a more positive redox potential ($E_{7.0}$ = 0.58 V) than tocopherol

($E_{7.0}$ = 0.48 V) and ascorbate ($E_{7.0}$ = 0.30 V) (Steenken et al. 1992). Stobadine enhanced consumption of chromanol-α-C6 and increased the magnitude of the chromanoxyl and ascorbyl radical electron spin resonance signal generated by lipoxygenase and arachidonate. These findings were interpreted as a result of the interaction of stobadinyl radicals with the chromanol ring and ascorbate (Kagan et al. 1993). In addition, trolox, a water-soluble analogue of α-tocopherol, markedly reduced dissipation of stobadine in a system consisting of liposomal membrane and the peroxyl radical generation inducing azoinitiator 2,2'-azobis-(2-amidinopropane) hydrochloride (Rackova et al. 2002).

Contrary to its effect on NCV, the therapy used in our experiments was ineffective on diabetes-induced RICF changes. The mechanisms underlying RICF are controversial and has not been fully explained yet.

RICF can be produced acutely by an oral glucose load and corrected by insulin treatment. RICF therefore may be related to the metabolic availability of high glucose (Baba et al. 2006).

Another hypothesis suggests that it is a polyol-pathway-related consequence of reduced Na,K-ATPase activity; hence the diminished demand for ATP and oxygen (Lattimer et al. 1989). Nerve function could also be adversely affected by hyperglycaemic pseudohypoxia caused by excessive flux through the second half of the polyol pathway, which may promote an elevation in cell NADH/NAD ratio with widespread effects on intermediate metabolism (Williamson et al. 1993). The vascular hypothesis attributes hypoxic resistance to adaptation to the hypoxic endoneurium, involving increased reliance on anaerobic energy metabolism (Low et al. 1989; Cameron and Cotter 1994). Results from vasodilatator treatment experiments showed that RICF could be prevented without any effect on nerve polyol levels (Cameron et al. 1992). Although the combination of stobadine and vitamin E reduced nerve conduction slowing probably by partially increasing nerve perfusion and reducing endoneurial hypoxia, these measures may not be sufficient to prevent hypoxic resistance. RICF is an apparently more sensitive indicator of diabetic nerve dysfunction than NCV and correspondingly more difficult to correct (Cameron et al. 1992). In addition, hyperglycaemic exposure can independently stimulate nerve anaerobic metabolism (Strupp et al. 1991) and our intervention had no effect on the severity of diabetes.

In conclusion, the present experiments showed that monotherapy with stobadine as well as with the combination of stobadine and vitamin E in the given dosage regimen partially protected against NCV deterioration. This is in agreement with the finding that the antioxidant potency of stobadine may be increased by its interaction with antioxidants with more negative redox potentials. The results indicate that some antioxidants may be partially effective against nerve dysfunction on the basis of attenuating the enhanced oxidative stress that correspond with chronic hyperglycaemia within diabetic conditions.

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