doi: 10.1111/j.1472-8206.2004.00312.x

ORIGINAL ARTICLE

Reactive oxygen species mediate abnormal contractile response to sympathetic nerve stimulation and noradrenaline in the vas deferens of chronically diabetic rats: effects of in vivo treatment with antioxidants

A. Güneş[a], A. Ceylan[b], Y. Sarıoğlu[a], M. Stefek[c], V. Bauer[c], Ç. Karasu[a]*, The Antioxidants in Diabetes-induced Complications (ADIC) Study Group

[a]*Department of Medical Pharmacology, Faculty of Medicine, Gazi University, Ankara, Turkey*
[b]*Department of Pharmacology, Faculty of Pharmacy, Ankara University, Ankara, Turkey*
[c]*Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovakia*

Keywords
neuroadrenergic stimulation,
rat,
stobadine,
streptozotocin-diabetes,
superoxide,
vas deferens,
vitamin E

Received 18 October 2003;
revised 5 March 2004;
accepted 7 October 2004

*Correspondence and reprints:
karasu@gazi.edu.tr

ABSTRACT

Previous studies suggest that a link exists between increased oxidative stress and diabetic neuropathy. Moreover, antioxidants may protect neurones from the degenerative effects of reactive oxygen species. In our study, we used streptozotocin (STZ)-diabetic rats in a 8-month chronic diabetes model to study the effects of in vivo treatment with stobadine (ST), a pyridoindole antioxidant, and vitamin E. STZ-diabetic rats were treated with ST (24.7 mg/kg/day), vitamin E (D,L-alpha-tocopheryl acetate, 400–500 IU/kg/day) or ST plus vitamin E through an intra-oral catheter for a 8-month period beginning 10 days after STZ injection. Blood glucose and HbA1c levels were increased in diabetic rats by about 400 and 100%, respectively. Antioxidant treatment significantly decreased haemoglobin glycosylation ($P < 0.05$). We also determined the effects of chronic diabetes on sympathetic neurotransmission by measuring the contractility of isolated vas deferens. Furthermore, we investigated contractions elicited by electrical field stimulation (EFS) (1–64 Hz) which were significantly decreased in diabetic rats when compared with control rats. Treatment with ST or vitamin E alone partly enhanced the amplitude of the contractions induced by EFS, but a combination of ST and vitamin E treatment showed no additional effects. Contractile response of the vas deferens to exogenous noradrenaline, was increased in diabetic rats when compared with control rats. While the addition of vitamin E alone had no effect, ST completely returned noradrenaline-induced contractions to basal levels. The tension induced by 120 mM KCl was not statistically different among the experimental groups. In normal rats, EFS-induced contractions were significantly inhibited by pyrogallol (10^{-4} M), a free-radical generator. Percentage inhibition of pyrogallol on EFS (32 Hz)-induced contractions in ring sections was 48 ± 5.8 in control, 75 ± 5.5 in untreated-diabetic, 54 ± 2.7 in ST-treated diabetic, and 58 ± 4.7 in vitamin E-treated diabetic rats. Combining both ST and vitamin E treatment had the same effects as each antioxidant alone with a percent inhibition of 48 ± 6.8. These results are consistent with the degenerative changes seen in sympathetic nerves and the abnormal function observed in chronically diabetic rats, leading to a decrease in EFS response and an increase in response to adrenergic agonists in the vas deferens. Furthermore, we demonstrated that reactive oxygen species are responsible for impaired sympathetic neurotransmission and abnormal function of diabetic vas deferens, and that a combination of antioxidants may be better for the therapy of reproductive system disabilities in male diabetics.

INTRODUCTION

Neuropathy, is a common complication of diabetes mellitus, which has been shown to occur in the reproductive system in male patients with long-term diabetes mellitus [1]. Common neuropathic complications like erectile impotency [2], retrograde ejaculation [2], and decreased fertility [3] have been reported in diabetic patients. The effects of diabetes on sympathetic innervation and the function of vas deferens have been widely studied, but the findings obtained in animal models of diabetes are somewhat controversial. Previous findings show that rats with streptozotocin (STZ)-induced diabetes exhibit pathological changes in the noradrenergic nerves supplying the vas deferens [4]. The vas deferens of long-term experimental diabetic rats have been reported to display an impaired response to stimulation of their noradrenergic nerves and a hypersensitivity to exogenous noradrenaline, phenylephrine, ATP and acetylcholine [5,6]. The responsiveness of the vas deferens to α-adrenergic agonists and to sympathetic nerve stimulation is increased in short-term diabetes, but decreased in long-term diabetes [6,7]. Furthermore, an increase in the activity in both voltage-dependent and receptor-operated calcium channels and Ca^{2+} influx was also shown to contribute to the potentiation of contractile responses in diabetic vas deferens [8]. The acceleration of phosphatidylinositol turnover [8,9] or decreased calmodulin levels was reported to account for the abnormal responsiveness of the vas deferens in long-term STZ-diabetic rats [7]. It has been demonstrated that the degeneration of sympathetic neurones, which occurs in the vas deferens in 10-week STZ-diabetic rats, is characterized by a greater amplitude in the spontaneous junction potentials linked to an increase in Ca^{2+} mobilization [10].

On the contrary, the positive relationship between increased oxidative stress and functional, structural and biochemical abnormalities in the autonomic nervous system have already been reviewed in detail in experimentally diabetic animals [11–13]. Treatment with different antioxidants may prevent or reverse abnormal nerve function and biochemistry, in addition to protecting neurones from the degenerative effects of reactive oxygen species [11,14,15]. However, neither the effects of chronic diabetes over an 8-month period nor the effects of treatment with antioxidants, has been studied in terms of sympathetic neurotransmission and contractile function of the vas deferens. Thus, the objectives of the present study were: (i) to clarify the effects of chronic (8-month) diabetes on the sympathetic neurotransmission and the contractile response of the vas deferens, and (ii) to investigate the effects of in vivo treatment with different antioxidants: vitamin E and stobadine, a pyridoindole compound.

RESEARCH DESIGN AND METHODS

Induction of diabetes and treatment protocols

Male Wistar rats, body weight 250–300 g, were fed a standard rat chow diet and had access to water ad libitum. Diabetes was induced by a single intraperitoneal injection of STZ (55 mg/kg body weight) in animals that were made to fast overnight. Diabetes was verified 48 h later by measuring tail vein blood glucose, and rats with blood glucose levels of 300 mg/dL or more were considered diabetic. Ten days after the injection of STZ or vehicle, the rats were divided into the following groups: (i) untreated diabetic; (ii) diabetic treated with ST (24.7 mg/kg/day, orally); (iii) diabetic treated with vitamin E (D,L alpha-tocopheryl acetate, 400–500 IU/kg/day, orally); (iv) diabetic treated with both ST and vitamin E, as in protocols (ii) and (iii); and (v) untreated control rats. The animals were treated for a period of 8 months beginning 10 days after either vehicle or STZ injection. The principles of laboratory animal care (NIH publication no. 85–23, revised 1985) were observed.

Blood glucose and HbA1c

Blood glucose concentrations were measured with an Accutrend® GCT meter (Roche Diagnostics, Mannheim, Germany). HbA1c levels were determined at the end of the experiments as described previously [16].

Isolated rat vas deferens and the experimental protocols

Both vas deferens were carefully removed with a portion of the prostate and placed in a Petri dish filled with fresh Krebs' solution (pH 7.4) consisting of (in mM): NaCl 118.4, KCl 4.7, $CaCl_2$1.9, $NaHCO_3$ 25.0, $MgSO_4{\cdot}7H_2O$ 1.2, KH_2PO_4 1.2, and glucose 11.1. A 1-cm section was taken from the prostate end of the vas deferens. The tissues were suspended in an organ bath containing 20 mL Krebs' solution. The solution was maintained at 37 °C and bubbled with 95% O_2–5% CO_2. The resting tensions were adjusted to 1.0 g and the contractility was recorded for each tissue with an isometric transducer (Ugo Basile, No. 7004) (Varese, Italy) connected to a recorder (Ugo Basile, No. 7050) according to a previously

described method [17]. Electrical field stimulation (EFS) was produced by bipolar platinum electrodes placed around the strips. A Grass stimulator (S-48, Quincy, MA, USA) was used to generate single square pulses of 50 V for 0.5 ms, and the responses to EFS (1–64 Hz) were recorded. The contractions elicited by EFS were expressed as a percentage of the maximal response induced by 120 mM KCl for each vas deferens preparation. After washing twice with a 20-min interval between washes, the contractions of isolated vas deferens in response to noradrenaline were measured. To investigate the role of free radicals, EFS (32 Hz)-induced contractions were determined in the presence of 10^{-4} M concentration of pyrogallol, a superoxide generator.

Analysis of data and statistics

Data were expressed as mean ± SEM. Responses to EFS and noradrenaline were measured in g tension and expressed as a percentage of contractile response to 120 mM K^+. pD_2 values (negative logarithm of the molar concentration at which one maximal contraction occurs) were determined from individual concentration–response curves by nonlinear regression analysis. The responses obtained in each subject were averaged to yield a single value and therefore, all *n* values represent the number of subjects. Differences between untreated and treated groups were assessed by analysis of variance (ANOVA) followed by the Tukey's test. Statistical significance was accepted at $P < 0.05$. All analysis were performed using GraphPad statistical software (Graphpad, San Diego, CA, USA).

RESULTS

Blood glucose and HbA1c levels

Following 8 months of chronic diabetes, the glucose levels in untreated-diabetic rats were increased by about 400% and the HbA1c levels were increased by 100% (*Table I*), indicating high levels of glycosylation. When the diabetic animals were treated with antioxidants for 8 months, significant decreases in blood glucose and HbA1c concentrations were observed (*Table I*).

EFS, concentration–response studies and the effects of pyrogallol

The EFS of rat vas deferens elicited reproducible, frequency-dependent, contractile activity, which was significantly decreased in diabetic rats at frequencies from 1–64 Hz (*Figure 1*). ST treatment partially

Table I Final blood glucose and HbA1c concentrations in rats.

	Control ($n = 10$)	Untreated-diabetic ($n = 8$)	ST treated-diabetic ($n = 12$)	Vitamin E treated-diabetic ($n = 5$)	ST plus vitamin E treated-diabetic ($n = 8$)
Blood glucose (mg/dL)	112.0 ± 7.0**	504.3 ± 6.4*	413.2 ± 18.6*,**	384.4 ± 7.4*,**	390.8 ± 3.4*,**
HbA1c (%)	4.96 ± 0.19**	7.10 ± 0.09*	6.34 ± 0.14*,**	5.30 ± 0.17**,***	5.20 ± 0.15**,***

Data are reported as mean ± SEM.

*$P < 0.05$ vs. control; **$P < 0.05$ vs. untreated-diabetic; ***$P < 0.05$ vs. ST treated-diabetic.

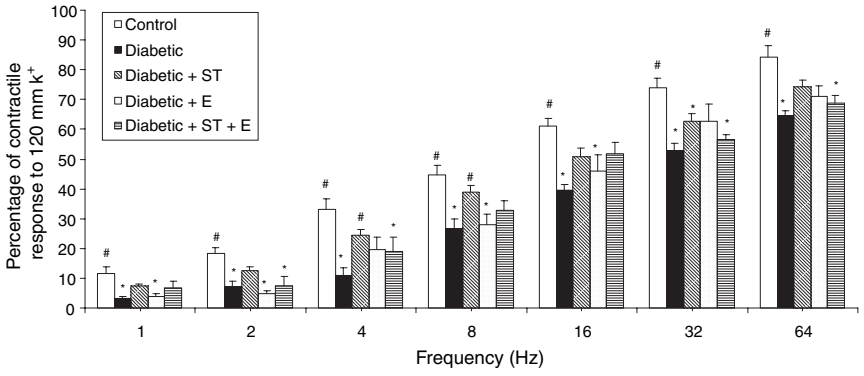

Figure 1 Contractile response of vas deferens to electrical field stimulation (EFS) in control and STZ-diabetic rats untreated or treated with stobadine (ST), vitamin E (E) or ST plus E. Data (mean ± SEM) were analysed by ANOVA and between-group differences for each variable were tested using Tukey's test. *$P < 0.05$ statistically different from control. #$P < 0.05$ statistically different from untreated diabetic.

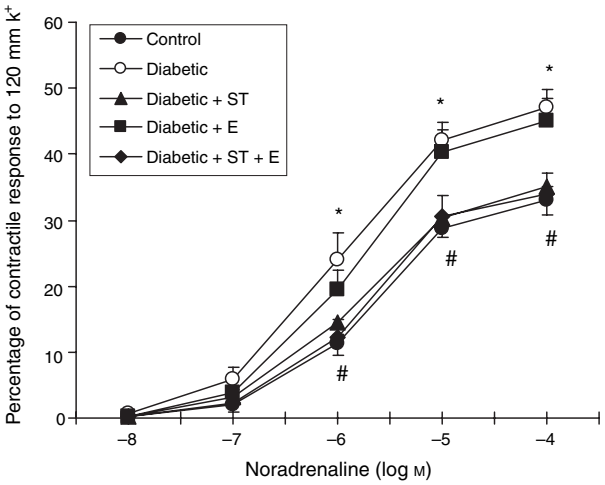

Figure 2 Contractile response of vas deferens to noradrenaline in control and STZ-diabetic rats untreated or treated with stobadine (ST), vitamin E (E) or ST plus E. Data (mean ± SEM) were analysed by ANOVA and between-group differences for each variable were tested using Tukey's test. $^{*}P < 0.05$ statistically different from control. $^{\#}P < 0.05$ statistically different from untreated diabetic.

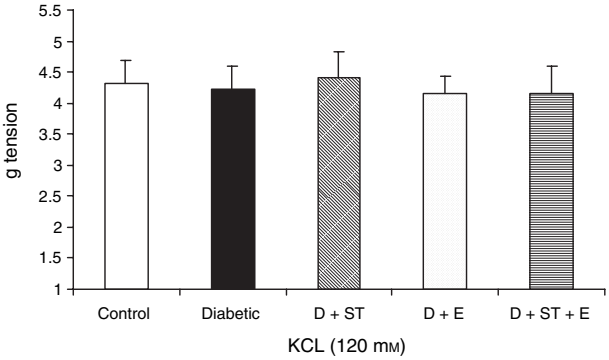

Figure 3 Contractile response of vas deferens to 120 mM KCl in control and STZ-diabetic rats untreated or treated with stobadine (ST), vitamin E (E) or ST plus E. Data (mean ± SEM) were analysed by ANOVA and between-group differences for each variable were tested using Tukey's test.

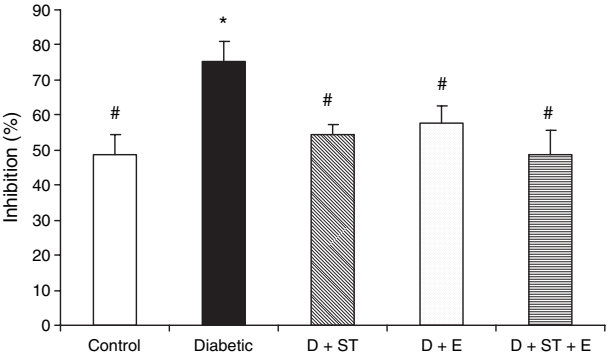

Figure 4 Percent inhibition in contractile response of vas deferens to electrical field stimulation at 32 Hz in the presence of 10^{-4} M pyrogallol in control and STZ-diabetic rats untreated or treated with stobadine (ST), vitamin E (E) or ST plus E. Data (mean ± SEM) were analysed by ANOVA and between-group differences for each variable were tested using Tukey's test. $^{*}P < 0.05$ statistically different from control. $^{\#}P < 0.05$ statistically different from untreated diabetic.

restored EFS-induced contraction amplitudes at all frequencies studied. Vitamin E treatment weakly enhanced EFS-induced contractility in diabetic rats. Combined effects of ST and vitamin E treatment did not improve the EFS-induced contractions in diabetic vas deferens over that observed for ST treatment alone (*Figure 1*).

The concentration–response curve for the contractile response of the vas deferens to exogenous noradrenaline in normal rats, is shown in *Figure 2*. Cumulative addition of noradrenaline (10^{-8} to 10^{-4} M) induced concentration-dependent contractions with a pD2 value of 5.8 ± 0.09. In rats with chronic diabetes, an increase in maximum contractile response of the rat vas deferens to noradrenaline, was observed. Vitamin E treatment alone was unable to reduce the maximum contractile response to noradrenaline in diabetic rats, but ST treatment brought it down to control levels (*Figure 2*). pD_2 values for noradrenaline-induced contractions were not statistically different among the groups (control = 5.8 ± 0.09; untreated-diabetic = 6.1 ± 0.4; ST treated-diabetic = 5.9 ± 0.4; vitamin E treated-diabetic = 5.7 ± 0.5; ST plus vitamin E treated-diabetic =5.8 ± 0.1). The tension induced by 120 mM KCl was not found to be statistically different among the experimental groups (*Figure 3*).

In normal rats, EFS-induced contractions were significantly inhibited by pyrogallol (10^{-4} M), a free-radical generator (% inhibition = 48 ± 5.8). An increase in the percent inhibition induced by pyrogallol was observed in untreated-diabetic rats (75 ± 5.5%). This was brought back to control levels by ST treatment (54 ± 2.7%) and by vitamin E treatment (58 ± 4.7%) in diabetic rats. A combination of both antioxidants showed a similar degree of protection from pyrogallol inhibition as was obtained with each agent alone (48 ± 6.8%) (*Figure 4*).

DISCUSSION

Our results show that there was a significant decrease in the response to EFS and a pronounced increase in

noradrenaline-induced contractility in the vas deferens of chronically diabetic rats. These results are in agreement with previous observations showing that STZ-diabetic rats exhibit a decrease in the responsiveness of the vas deferens to nerve stimulation and a non-specific supersensitivity to contractile agents [5–8,10]. These observations obtained from STZ-induced diabetic rats, however, were not confirmed in the vas deferens from spontaneously diabetic BB rats [18] nor in long-term alloxan diabetic rats [7]. The increased α-adrenergic responsiveness of diabetic vas deferens observed in this study, may be attributable to new receptor synthesis or activation of post-receptor events due to diabetes-induced neuropathy rather than an enhancement in α-adrenoceptor affinity, as the pD_2 for the contractile effects of noradrenaline was found to be unchanged. This is in agreement with the results of other groups [5,7,19]. The increased contractile responses of vas deferens from diabetic rats to phenylephrine, and the decreased responses to nerve stimulation in diabetic rats, has been explained with denervation-like supersensitivity in receptor-mediated processes [6]. Alternatively, the increased α-adrenergic responsiveness may be closely related to an increase in calcium release from the sarcoplasmic reticulum of diabetic vas deferens via an acceleration of phosphatidylinositol turnover and an increase in the basal activity of diacylglycerol kinase, although we found that cellular calcium entry via KCl sensitive channels is not changed by diabetes nor by antioxidant treatment in rat vas deferens. It has also been suggested that impaired intracellular calcium metabolism is associated with functional complications of vas deferens in diabetic rats [8–10].

Diabetes-induced oxidative stress is clearly present in the peripheral nerve, dorsal root, and sympathetic ganglia of the peripheral nervous system in diabetic animals. Endothelial cell dysfunction is implicated in nerve blood flow and conduction deficits, impaired neurotrophic support, changes in signal transduction and metabolism, and morphological abnormalities, which are all characteristic of peripheral diabetic neuropathy [1,4,20]. Previous studies indicate that increased generation of superoxide might be responsible for the development of diabetic vascular and neural complications [11–15,21]. We demonstrated that increased inhibition of contractile response of diabetic vas deferens to EFS by pyrogallol, a potent superoxide generator [22], involves the increased susceptibility of adrenergic nerves in the vas deferens to the destructive effects of reactive oxygen species in diabetic rats. The strong effects of pyrogallol in diabetic vas deferens, may be a consequence of decreased superoxide dismutase activity, which has been demonstrated previously in some peripheral nerves depending on the duration of hyperglycaemia [12]. Superoxide dismutase has been shown to have a crucial role in local defense mechanisms against tissue damage mediated through superoxide anion radicals in male genital organs [23]. When given separately to diabetic animals, antioxidants, ST and vitamin E, successfully reverse the inhibitory effects of pyrogallol in vitro. This can be explained by the ability of ST [24] and vitamin E [11,15,25] to inhibit the production of superoxide or to scavenge reactive oxygen species in diabetic tissues. Treatment with ST or vitamin E alone enhanced the amplitude of the contractions induced by EFS stimulation, but the effects of these antioxidants was partial and fluctuated depending on the frequency applied. No additive effects were observed by the combined treatment of ST and vitamin E in EFS-induced contractions in diabetic vas deferens compared with each antioxidant alone. Treatment of diabetic rats with vitamin E alone did not affect noradrenaline-stimulated contraction, but, on the contrary, ST brought the contractions in the vas deferens back to control levels when given alone or together with vitamin E. ST is known to be a potential protector of the central nervous system in diseases where oxidative injury plays an important role, such as stroke, neurotrauma, chronic brain ischaemia, and some neurodegenerative diseases [26,27]. Our study demonstrated that ST protected peripheral adrenergic nerves in rat vas deferens against diabetes-induced oxidative stress.

We previously demonstrated that vitamin E treatment ameliorated sciatic nerve function [14], which is reduced in rats with STZ-diabetes [14], and prevented or reversed abnormal contractility and endothelial dysfunction in acute or long-term diabetic rats [28]. The mechanisms of cellular effects of ST or vitamin E in the present study, may be linked to intracellular calcium metabolism as we demonstrated that these antioxidants are able to prevent calcium overload, lipid peroxidation, and protein glycation, as well as inhibit hyperglycemia-induced oxidative stress [29]. In addition, long-term vitamin E deficiency has been shown to cause dysfunction of autonomic neuroeffector mechanisms in the smooth muscle of rat vas deferens, at both a pre- and post-junctional level [11]. In diabetes, an increase in these abnormalities in neuroeffector systems in addition to the characteristic vitamin E deficiency observed in diabetes [12,30], can be lethal as previously shown in vitamin E-deficient STZ-diabetic animals [30].

These results confirm that sympathetic nerve endings show degenerative changes characterized by a decreased response to EFS and an increased response to adrenergic agonists in rat vas deferens. Moreover, reactive oxygen species are responsible for impaired sympathetic neurotransmission and the abnormal function of the vas deferens. Overall, a combination of antioxidants may prove to be therapeutic in reproductive system disability in male diabetics.

ACKNOWLEDGEMENTS

This study was supported by Scientific and Technical Research Council of Turkey [SBAG-2397 and SBAG-SLOVAK-2 (103S180)] and Ankara University Research Foundation (2001–0803031).

REFERENCES

1 Dunsmuir W.D., Holmes S.A. The aetiology and management of erectile, ejaculatory, and fertility problems in men with diabetes mellitus. Diabet. Med. (1996) **3** 700–708.
2 Richardson D., Vinik A. Etiology and treatment of erectile failure in diabetes mellitus. Curr. Diabet. Rep. (2002) **2** 501–509.
3 Zangen S.W., Yaffe P., Shechtman S., Zangen D.H., Ornoy A. The role of reactive oxygen species in diabetes-induced anomalies in embryos of Cohen diabetic rats. Int. J. Exp. Diabet. Res. (2002) **3** 247–255.
4 Tomlinson D.R., Gillon K.R., Smith M.G. Axonal transport of noradrenaline and noradrenergic transmission in rats with streptozotocin-induced diabetes. Diabetologia (1982) **22** 199–204.
5 Peredo H., Agostini M.D., Gimeno M.F., Borda E.S. Hypersensitivity to norepinephrine in vasa deferentia from diabetic rats. Possible participation of metabolic products of arachidonic acid. Prostaglandins Leukot. Med. (1984) **15** 229–239.
6 Longhurst P.A. The effects of testosterone or insulin treatment on contractile responses of the rat vas deferens following castration or streptozotocin-induced diabetes mellitus. Gen. Pharmacol. (1990) **21** 427–434.
7 Ozturk Y., Yıldizoglu-Ari N., Altan V.M. Altered alpha-adrenergic responses of vas deferens to noradrenaline and tyramine from rats with short- and long-term alloxan diabetes. Gen. Pharmacol. (1994) **25** 1519–1524.
8 Sakai Y., Honda H. Hyperactivity of Ca channels in vasa deferentia smooth muscle of diabetic rats. Pharmacol. Biochem. Behav. (1987) **27** 227–229.
9 Nobe K., Sakai Y., Momose K. Alternations of diacylglycerol kinase in streptozotocin-induced diabetic rats. Cell. Signal. (1998) **10** 465–471.
10 Kamata K., Kirisawa H. Changes in electrophysiological properties and noradrenaline response in vas deferens of diabetic rats. Eur. J. Pharmacol. (1998) **350** 237–241.
11 Nickander K.K., Schmelzer J.D., Rohwer D.A., Low P.A. Effect of alpha-tocopherol deficiency on indices of oxidative stress in normal and diabetic peripheral nerve. J. Neurol. Sci. (1994) **126** 6–14.
12 Van Dam P.S., Van Asbeck B.S., Bravenboer B., Van Oirschot J.F., Marx J.J., Gispen W.H. Nerve conduction and antioxidant levels in experimentally diabetic rats: effects of streptozotocin dose and diabetes duration. Metabolism (1999) **48** 442–447.
13 Obrosova I.G. How does glucose generate oxidative stress in peripheral nerve? Int. Rev. Neurobiol. (2002) **50** 3–35.
14 Karasu C., Dewhurst M., Stevens E.J., Tomlinson D.R. Effects of anti-oxidant treatment on sciatic nerve dysfunction in streptozotocin-diabetic rats; comparison with essential fatty acids. Diabetologia (1995) **38** 129–134.
15 Albino-Teixeira A., Azevedo I., Martel F., Osswald W. Superoxide dismutase partially prevents sympathetic denervation by 6-hydroxydopamine. Naunyn. Schmiedebergs Arch. Pharmacol. (1991) **344** 36–40.
16 De Tata V., Novelli M., Bombara M. et al. Determination of glycated hemoglobins in the rat: comparison between two different chromatographic methods and application in experimental diabetology. Res. Exp. Med. (Berl.) (1996) **196** 9–16.
17 Banchelli G., Raimondi L., Ghelardini C. et al. Benzylamine-related compounds stimulate rat vas deferens neurotransmission and potentiate memory in the mouse acting as potassium channel blockers. Pharmacol. Res. (2000) **41** 151–162.
18 Longhurst P.A. In vitro contractile responses of vasa deferentia from spontaneously diabetic BB rats. J. Auton. Pharmacol. (1991) **11** 63–71.
19 Peredo H.A. Prostanoid release and constrictor responses to noradrenaline in the rat mesenteric vascular bed in non-insulin-dependent diabetes mellitus. J. Auton. Pharmacol. (2001) **21** 131–137.
20 Tomlinson D.R. Future prevention and treatment of diabetic neuropathy. Diabetes Metab. (1998) **24** (Suppl 3), 79–83.
21 Coppey L.J., Gellett J.S., Davidson E.P., Dunlap J.A., Lund D.D., Yorek M.A. Effect of antioxidant treatment of streptozotocin-induced diabetic rats on endoneurial blood flow, motor nerve conduction velocity, and vascular reactivity of epineurial arterioles of the sciatic nerve. Diabetes (2001) **50** 1927–1937.
22 Lopez B.L., Liu G.L., Christopher T.A., Ma X.L. Peroxynitrite, the product of nitric oxide and superoxide, causes myocardial injury in the isolated perfused rat heart. Coron. Artery Dis. (1997) **8** 149–153.
23 Nonogaki T., Noda Y., Narimoto K. et al. Localization of CuZn-superoxide dismutase in the human male genital organs. Hum. Reprod. (1992) **7** 81–85.
24 Horakova L., Stolc S. Antioxidant and pharmacodynamic effects of pyridoindole stobadine. Gen. Pharmacol. (1998) **30** 627–638.
25 Sies H., Stahl W., Sundquist A.R. Antioxidant functions of vitamins. Vitamins E and C, beta-carotene, and other carotenoids. Ann. N.Y. Acad. Sci. (1992) **669** 7–20.
26 Stolc S., Selecka J. Protective effect of stobadine, a pyridoindole antioxidant, in hypoxia-reoxygenation injury of ganglionic and

hippocampal neurotransmission. Mol. Chem. Neuropathol. (1995) **25** 199–212.

27 Stolc S., Vlkolinsky R., Pavlasek J. Neuroprotection by the pyridoindole stobadine: a minireview. Brain Res. Bull. (1997) **42** 335–340.

28 Karasu C., Ozansoy G., Bozkurt O., Erdogan D., Omeroglu S. Antioxidant and triglyceride-lowering effects of vitamin E associated with the prevention of abnormalities in the reactivity and morphology of aorta from streptozotocin-diabetic rats. Antioxidants in Diabetes-Induced Complications (ADIC) Study Group. Metabolism (1997) **46** 872–879.

29 Pekiner B., Ulusu N.N., Das-Evcimen N. et al. Antioxidants in Diabetes-Induced Complications Study Group. In vivo treatment with stobadine prevents lipid peroxidation, protein glycation and calcium overload but does not ameliorate Ca^{2+}-ATPase activity in heart and liver of streptozotocin-diabetic rats: comparison with vitamin E. Biochim. Biophys. Acta (2002) **1588** 71–78.

30 van Dam P.S., van Asbeck B.S., Bravenboer B., van Oirschot J.F., Gispen W.H., Marx J.J. Nerve function and oxidative stress in diabetic and vitamin E-deficient rats. Free Radic. Biol. Med. (1998) **24** 18–26.