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Effects of vitamin E and sodium selenate on neurogenic and endothelial relaxation of corpus cavernosum in the diabetic mouse

Cemil Göçmen*, Ata Seçilmiş, Eda Karabal Kumcu, Peyman Uçar Ertuğ, Serpil Önder, Atilla Dikmen, Firuz Baysal

Department of Pharmacology, Medical Faculty, Çukurova University, TR-01330 Adana, Turkey

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Abstract

We studied the effect of vitamin E and sodium selenate treatment on the neurogenic and endothelium-dependent relaxation of isolated corpus cavernosum obtained from streptozotocin-induced diabetic mice. Relaxant responses of corpus cavernosum precontracted by phenylephrine to electrical field stimulation and to acetylcholine were significantly decreased in diabetic mice. There was no significant difference between diabetic and non-diabetic groups for the relaxant response of corpus cavernosum to sodium nitroprusside and papaverine. Treatment with sodium selenate, but not vitamin E, partially prevented the impairment of the neurogenic relaxation, whereas both had a significant, partial restorative action on endothelial dysfunction in corpus cavernosum obtained from diabetic groups. Neither agent exhibited a significant action on the relaxant responses of corpus cavernosum obtained from non-diabetic mice. A decrease in the sensitivity of the neurogenic impairment to antioxidant action may develop more rapidly than that of endothelial dysfunction in streptozotocin-induced diabetic mice.

Keywords: Streptozotocin-induced diabetic mouse; Corpus cavernosum; Nitric oxide (NO); Antioxidant; Vitamin E; Sodium selenate; Oxidative stress

1. Introduction

The neurological and vascular complications of diabetes are presumed to be responsible for the erectile dysfunction (Saenz de Tejada et al., 1989). It has been demonstrated that neurogenic and endothelium-mediated relaxation of the cavernous tissue was impaired in diabetic men with impotence and animal models of diabetes (Saenz de Tejada et al., 1989; Azadzoi and Saenz de Tejada, 1992; Bemelmans et al., 1994; Keegan et al., 1999). Recent evidence suggests that oxidative injury may play a role in the development of neurological and vascular complications of diabetes (Azadzoi and Saenz de Tejada, 1992; Baynes, 1991; Low and Nickander, 1991; Stohs, 1995; Cameron and Cotter, 1997). It has been reported that oxidative stress is present as early as 1 month after the onset of diabetes (Low and Nickander, 1991; Low et al., 1997; Kakkar et al., 1997). Several factors promote oxidative stress in diabetes, including increased free radical production (Baynes, 1991; Low and Nickander, 1991; Stohs, 1995) and impairment of the tissue anti-oxidant protection system (Baynes, 1991; Cameron et al., 1993; Low et al., 1997). Beneficial effects of some antioxidants, such as α-lipoic acid, vitamin E, and sodium selenate on nerve and endothelial dysfunction due to oxidative stress have been observed in animals with diabetes (Cameron et al., 1993; Kahler et al., 1993; Keegan et al., 1995; Douillet et al., 1996; Low et al., 1997). In a recent study, it was shown that α-lipoic acid showed prevention and partial correction of the impairment of nitrergic relaxations, largely dependent on nitric oxide, of the corpus cavernosum in diabetic rats (Keegan et al., 1999). Based on this finding, to investigate the effect of antioxidant agents other than α-lipoic acid on the impairment in nitrergic relaxation of the corpus cavernosum from a diabetic species other than the rat might provide additional evidence for the effectiveness of the antioxidant treatment. Our previous study on the mouse corpus cavernosum revealed that electrical field stimulation- and acetylcholine-induced relaxations ap-

* Corresponding Author. Tel.: +90-322-338-6060 (3239); fax: +90-322-338-6572.

E-mail address: gocmencc@hotmail.com (C. Göçmen).

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peared to be mediated by a nitrergic mechanism, since they were completely abolished by N^G-nitro-L-arginine (10 μM), a nitric oxide synthase inhibitor (Göçmen et al., 1997). In the same study, disappearance of the electrical field stimulation-evoked relaxation was also demonstrated in the presence of tetrodotoxin (0.1 μM). Therefore, the mouse corpus cavernosum may be a useful preparation for the examination of both neurogenic and endothelium-dependent relaxation. In this context, the aim of the present study was to investigate the effects of antioxidant agents, vitamin E, and sodium selenate on the dysfunction of the corpus cavernosum caused by streptozotocin-induced diabetes in mice.

2. Materials and methods

2.1. In vivo experiments

Male albino mice (Wistar) weighing 30–32 g were housed separately according to their experimental groups in a room with controlled temperature and humidity, with a 12-h light–12-h dark photoperiod and received food and tap water ad libitum throughout the experiment. The mice were randomly divided into two main groups. In the non-diabetic control groups, the vehicle, 0.1 M citrate buffer (pH: 4.5) used to dissolve streptozotocin was intraperitoneally injected (0.3 ml) twice to all animals at a 24-h interval. Some mice were left free in their cages until they were killed for the determination of corpus cavernosum reactivity in vitro on the 20th or 30th day after the last vehicle injection. Others received either an intramuscular vitamin E (150 mg kg^{-1} day^{-1}) or an intraperitoneal sodium selenate (8 μg kg^{-1} day^{-1}) injection for 30 days, starting 24 h after the last injection of the vehicle. A blood sample was obtained from the tail vein immediately before killing of the animal for the in vitro experiment. Blood glucose levels were measured individually, using the glucose oxidase enzymatic assay (Randox Laboratories Antrim, UK). A total of 44 mice were used in the non-diabetic groups. In the second main group, a protocol similar to the above was used, except that all animals received intraperitoneal streptozotocin (100 mg/kg) together with the vehicle twice at a 24-h interval (Diabetic groups). All other procedures were the same as those for the non-diabetic group, except that some mice receiving vitamin E or sodium selenate were taken into in vitro experiments on the 20th day after the last streptozotocin injection to examine the time course of the effect of antioxidant treatment on corpus cavernosum reactivity, others were killed on the 30th day. In diabetic groups, six mice died within 30 days. Four mice were eliminated since their blood glucose levels were below 14 mmol/l. Thus, the remaining total of 46 mice was used in the diabetic groups.

2.2. In vitro experiments

The animals were killed by cervical dislocation. Penises were surgically removed and were then placed in a Petri dish containing Krebs solution (composition in mM: NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 15, $NaHPO_4$ 1.2, glucose 11). Corpus cavernosum was prepared according to a method previously described by Göçmen et al. (1997). Briefly, the fibrous septum between the two corpus cavernosum was cut and each corpus cavernosum was carefully dissected from the adherent tissues, keeping the tunica albuginea intact. The preparation was mounted between two platinum electrodes embedded in perspex under 0.2 g tension in an organ bath of 5 ml capacity containing Krebs solution. The bathing medium was continuously aerated with 95% O_2 and 5% CO_2 and its temperature was maintained at 37°C. The tissue was allowed to equilibrate for 1 h. The responses were recorded with isotonic transducers (Ugo Basile, 7006, Italy) on a two-channel recorder (Gemini 7070, Italy). After the equilibration period, phenylephrine (5 μM) was added into the bath. When the contraction reached steady state (within 5 min), acetylcholine or sodium nitroprusside was added to the bathing medium, or electrical field stimulation (10 V, 2 ms) delivered by a stimulator (Grass S88) was applied to the tissue for 30 s. After the response was recorded, the tissue was washed with fresh Krebs solution and allowed to rest for 10 min. Contact time of the tissue with each drug concentration was 1 min. This procedure was repeated for each acetylcholine (10^{-8}, 10^{-7} and 10^{-6} M) and sodium nitroprusside concentration (10^{-8}, 10^{-7} and 10^{-6} M) and each frequency (2, 5 and 10 Hz). At the end of the resting period, after all drugs and electrical field stimulation applications were completed, the tissue was contracted with phenylephrine once more, the relaxation induced by papaverine (5×10^{-4} M) was monitored and the experiment was terminated.

2.3. Drugs and solutions

Stock solutions of atropine sulphate, guanethidine, phenylephrine, acetylcholine, sodium nitroprusside, papaverine, and sodium selenate were dissolved in distilled water. Except vitamin E (α-tocopherol acetate, Ephynal ampule, 100 mg/2 ml, Roche), all drugs were obtained from Sigma Chemical.

2.4. Statistical considerations

Relaxations were calculated as percentage peak reduction of phenylephrine contracture. The mean values (±S.E.) for each groups were calculated separately. All data were evaluated in a one-way analysis of variance (ANOVA; Bonferroni-corrected *t*-test), using a computer

program, SPSS. P values of less than 0.05 were considered to be significant.

3. Results

3.1. Weight and plasma glucose levels in control and diabetic mice

Animals injected with streptozotocin had a significant weight loss compared to age-matched controls on the 20th day (not shown) and 30th day (Table 1). Treatment with vitamin E or sodium selenate had no significant effect on weight loss in the diabetic mice. Plasma glucose levels were significantly raised in the diabetic animals compared to the controls on the 20th (not shown) and 30th day (Table 1). No significant difference was observed in plasma glucose concentrations between vitamin E- or sodium selenate-treated and untreated diabetic mice on the 20th day (not shown) and 30th day (Table 1).

3.2. Relaxation induced by electrical field stimulation

The relaxant responses to all frequencies of electrical field stimulation (2, 5 and 10 Hz) were significantly decreased in the untreated diabetic mice compared to those in the normal control mice (Figs. 1A and 2). The neurogenic relaxation was significantly decreased on the 20th day of diabetes. This decrease was not significantly different from that observed on the 30th day. The impairment of the relaxation induced by electrical field stimulation in the diabetic mice could be significantly restored by sodium selenate treatment at the 20th day ($P < 0.05$ at 5 and 10 Hz) and the 30th day ($P < 0.05$ at 10 Hz) of diabetes (Fig. 2A and B), whereas vitamin E treatment failed to yield any significant effect on the impairment of relaxation (Fig. 2A and B). The restorative effect of sodium selenate was significantly decreased on the 30th day compared to that on the 20th day; the mean values ($\pm$S.E.) for percentage relaxation of the tissue at 5 and 10 Hz were 15.0 ± 1.0 versus 27.0 ± 3.0 ($P < 0.05$) and 27.0 ± 3.1 versus 39.6 ± 3.0 ($P < 0.05$), respectively. Vitamin E or sodium selenate treatment had no significant effect on the relaxation in response to electrical field stimulation in the control groups (not shown).

Table 1
Plasma glucose levels and weight of control and diabetic groups on the 30th day

	Plasma glucose levels (mmol/l)	Weight (g)
Control	7.44 ± 0.07 ($n = 10$)	31 ± 2 ($n = 10$)
Diabetic untreated	15.33 ± 0.08^a ($n = 8$)	20 ± 3^a ($n = 8$)
Diabetic vitamin E-treated	15.83 ± 0.05^a ($n = 7$)	18 ± 4^a ($n = 7$)
Diabetic selenium-treated	14.83 ± 0.07^a ($n = 8$)	22 ± 2^a ($n = 8$)

[a] Indicates significant differences from control groups ($P < 0.05$).

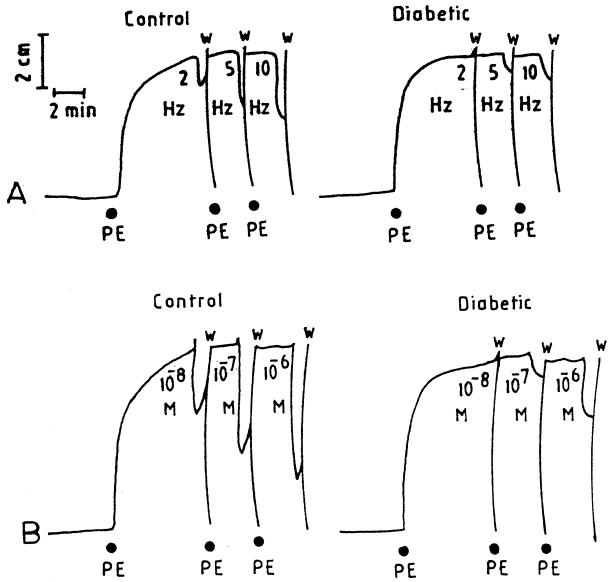

Fig. 1. Representative tracings of the relaxation of control and untreated diabetic mouse corpus cavenosum precontracted with 5×10^{-6} M phenylephrine (PE) on the 30th day, induced by (A) electrical field stimulation (EFS) and (B) acetylcholine (ACh); w: washout.

3.3. Relaxation induced by acetylcholine

Acetylcholine-induced relaxation at all concentrations was significantly attenuated in untreated diabetic mice

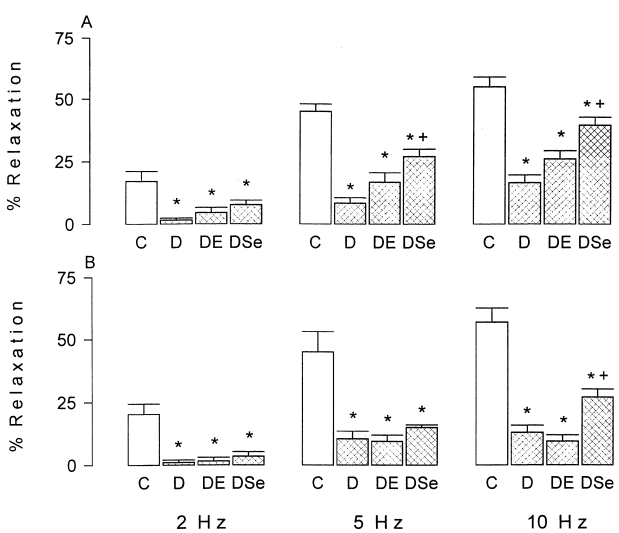

Fig. 2. The relaxant responses to electrical field stimulation in the mouse corpus cavernosum precontracted with 5×10^{-6} M phenylephrine (PE) from (C) control, (D) untreated diabetic, (DE) vitamin E-treated diabetic, and (DSe) sodium selenate-treated diabetic groups on the 20th day (A) and the 30th day (B) after the last streptozotocin or vehicle injection. Each column represents the mean relaxant response expressed as percentage peak reduction of phenylephrine contraction. (*) indicates significant differences in relaxation between all diabetic and control groups, (+) indicates significant differences in relaxation between untreated diabetic and vitamin E- or sodium selenate-treated diabetic groups for each frequency. * and + $P < 0.05$ ($n = 8$–15).

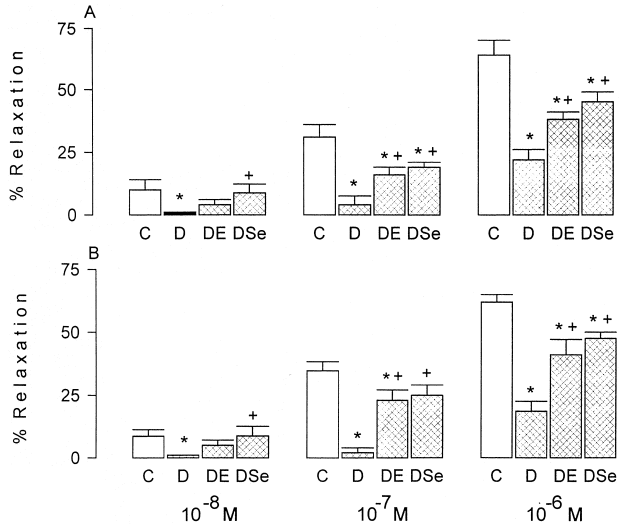

Fig. 3. The relaxant responses to acetylcholine in the mouse corpus cavernosum precontracted by 5×10^{-6} M phenylephrine from (C) control, (D) untreated diabetic, (DE) vitamin E-treated diabetic, and (DSe) sodium selenate-treated diabetic groups on the 20th day (A) and the 30th day (B) after the last streptozotocin or vehicle injection. Each column represents the mean relaxant response expressed as percentage peak reduction of phenylephrine contraction. (*) indicates significant differences in relaxation between all diabetic and control groups, (+) indicates significant differences in relaxation between untreated diabetic and vitamin E- or sodium selenate-treated diabetic groups for each concentration. * and + $P < 0.05$ ($n = 8$–15).

(Fig. 1B) and partially restored by treatment with either vitamin E ($P < 0.05$ at 10^{-7} and 10^{-6} M) or sodium selenate ($P < 0.05$ at all concentrations) at the 20th and the 30th days of diabetes (Fig. 3A and B). The anti-oxidant agents did not exhibit any significant action on the relaxation caused by acetylcholine in control groups (not shown).

3.4. Relaxation induced by sodium nitroprusside and papaverine

There was no significant difference between diabetic and control groups for the relaxation of corpus cavernosum induced by sodium nitroprusside (10^{-8}, 10^{-7} and 10^{-6} M) or 5×10^{-4} M papaverine at the 20th and 30th days (not shown).

4. Discussion

The major finding of the present study was that sodium selenate treatment has a partial restorative action on neuronal and endothelial dysfunction in corpus cavernosum obtained from streptozotocin-induced diabetic mice. On the other hand, vitamin E was ineffective on the impairment of neurogenic relaxation whereas it had a partial restorative action on endothelial dysfunction. Neither agent caused alterations of body weight or blood glucose level.

Previous studies have suggested that oxidative stress may play an important role in the early stage of experimental diabetic neuropathy and angiopathy by promoting auto-oxidation reactions which increase free radical production (Baynes, 1991; Low and Nickander, 1991). Some studies detected oxidative stress in rat peripheral nerves and kidney 2 weeks after the beginning of diabetes mellitus (Low and Nickander, 1991; Kakkar et al., 1997; Low et al., 1997). In these studies, neuropathy and nephropathy were also observed within 30 days. These findings support our results that neuronal and endothelial dysfunctions in the cavernous tissue were observed on the 20th day after the last streptozotocin injection. On the other hand, in the present study, there was no impairment in the relaxation mechanisms of the tissue, since papaverin-induced relaxation was not significantly different from the control. In addition, there was no reduction in the sensitivity of the tissue to nitric oxide since the relaxation induced by a nitric oxide donor, sodium nitroprusside, was not significantly different from that of tissue from non-diabetic mice, indicating that cGMP production is still intact. It is well known that nitric oxide has an important role in penile erection and is very sensitive to oxidative reactions. Therefore, it is possible that increased intracellular glycosylation products due to diabetes may impair the synthesis and availability of nitric oxide (Bucala et al., 1991; Azadzoi and Saenz de Tejada, 1992). However, it has been recently observed that nitric oxide synthase activity in anococcygeus muscles obtained from 8-week diabetic rats was not significantly different from that of non-diabetics (Way et al., 1999). If this is also the case for the mouse corpus cavernosum, it can be thought that at least in early stage of diabetes, increased catabolism of nitric oxide may be mainly responsible for the decrease in the nitrergic responses of the tissue. However, further studies based on measurement of nitric oxide synthase activity are needed for mouse corpus cavernosum. Beneficial effects of vitamin E and sodium selenate on the neuronal and endothelial dysfunction due to oxidative stress have been demonstrated in studies on experimental diabetes models (Cotter et al., 1995; Keegan et al., 1995; Douillet et al., 1996; Karasu et al., 1997). In the present study, only sodium selenate caused significant restoration of the neurogenic relaxation on the 20th day. On the 30th day, however, the restorative sodium selenate action had disappeared and had significantly decreased for the responses of the tissue to electrical field stimulation at 5 and 10 Hz, respectively, indicating that a decrease in the restorative sodium selenate action on the neurogenic impairment may develop with time. On the other hand, vitamin E did not display any significant restorative effect on the neuronal dysfunction. In previous studies on streptozotocin-induced diabetic rats, it had been observed that vitamin E supplementing failed to restore peripheral nerve function and that much higher vitamin E doses (1000 mg/kg) were necessary to obtain a preventive effect against sciatic nerve dysfunction

(Nickander et al., 1994; Cotter et al., 1995). A recent study on rat corpus cavernosum, however, showed that an antioxidant agent, α-lipoic acid, prevented and partially reversed the diabetic deficit in endothelium-dependent and neurogenic relaxation, respectively (Keegan et al., 1999). Regarding these findings, the ineffectiveness of vitamin E on neurogenic dysfunction in the present study may depend on the tissue under the study and/or on a relative difference between antioxidant actions of α-lipoic acid and vitamin E as well as on the dose used. However, vitamin E did exhibit a partial restorative action on the impairment of endothelium-dependent relaxation. A similar effect was also observed with sodium selenate. An interesting finding was that on the 30th day, the restorative actions of both agents on the endothelium-dependent relaxation were not less than those observed on the 20th day, indicating that the reduction in the effectiveness of sodium selenate on the neurogenic dysfunction action may develop more rapidly than that on the endothelial dysfunction. Beneficial effects possibly due to antioxidant activity of the agents used may be limited to the early stage of diabetes. In this period, they can protect nitric oxide against increased intracellular free radical actions (Giugliano et al., 1995; Green et al., 1995; Halliwell, 1995; Galley et al., 1997; Kinlay et al., 1999). However, further work is needed to determine glutathione levels as an index of antioxidant activity in the tissue used. On the other hand, vitamin E has not only an antioxidant effect, but also an independent inhibitory action on protein kinase C in vascular cells (Gökçe and Frei, 1996; Clement et al., 1997; Fazzio et al., 1997; Ricciarelli et al., 1998). Protein kinase C may play a role in corpus cavernosum dysfunction in diabetic mice and the restorative action of vitamin E on the endothelial impairment may be partially due to its inhibitory action on the enzyme (Keaney et al., 1996).

In conclusion, vitamin E or sodium selenate treatment may be of partial and transient benefit to the erectile function of corpus cavernosum from streptozotocin-induced diabetic mice. In the early stage of diabetes, an increased oxidative activity in the tissue may be a major reason for the diminution in effectiveness of the nitrergic mechanism. In this regard, agents with antioxidant properties may be useful to provide a partial improvement in the relaxant response by protecting nitric oxide from attack by oxidative products. However, the reduction in the effectiveness of sodium selenate on neurogenic dysfunction occurred within 10 days suggesting that, in spite of sodium selenate treatment, the nitrergic nerve impairment may be advancing.

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