Pharmacological Reports
2008, 60, 725–734
ISSN 1734-1140

Effects of ethanol treatment on the neurogenic- and endothelium-dependent relaxation of corpus cavernosum smooth muscle in the mouse

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Abstract:
The relaxation of cavernous smooth muscle is critical for inducing and maintaining a penile erection. The neurogenic- and endothelium-dependent relaxation of corpus cavernosum smooth muscle and the degenerative effect of subacute ethanol treatment on the endothelial cells of corpus cavernosum was investigated in mice. In the cavernous strips contracted with phenylephrine, electrical field stimulation (EFS), acetylcholine and exogenous nitric oxide (NO) induced relaxations in the control group. Ethanol treatment abolished the endothelium-dependent relaxations induced by acetylcholine but failed to alter the relaxation to EFS and NO. L-nitroarginine, a NO synthase inhibitor, reduced relaxations induced by EFS and acetylcholine, but not those induced by NO in control and ethanol-treated mice. L-arginine prevented the response inhibited by L-nitroarginine. ODQ, a guanylyl cyclase inhibitor, inhibited relaxations in response to EFS, NO and acetylcholine in control and ethanol-treated mice. Corpus cavernosum tissues were investigated using electron microscopy and endothelial damage was observed in ethanol-treated mice. These results suggest that ethanol impairs the endothelial function of corpus cavernosum in mouse, and it may lead to erectile dysfunction through a reduced NO release *via* endothelial impairment.

Key words:
corpus cavernosum, ethanol, endothelium, neurogenic-and-endothelium-dependent relaxation, nitric oxide

Abbreviations: cGMP – cyclic guanosine 3,5-monophosphate, EFS – electrical field stimulation, eNOS – endothelial nitric oxide synthase, iNOS – inducible NOS, NO – nitric oxide, NOS – nitric oxide synthase, nNOS – neuronal NOS, ODQ – 1 *H*-[1,2,4]oxadiazolol[4,3-*a*]quinoxalin-1-one

Introduction

Penile erection is a complex neurovascular process that involves relaxation of the corpus cavernosum smooth muscle [2]. Many studies have shown that nitric oxide (NO) released from cavernous nerves and endothelium is a key factor necessary for penile erection. Released NO activates soluble guanylate cyclase and increases the production of cyclic guanosine 3,5-monophosphate (cGMP) [37]. NO is formed from the conversion of L-arginine by nitric oxide synthase (NOS), which exists in three isoforms: neuronal (nNOS), endothelial (eNOS) and inducible (iNOS). nNOS is expressed in penile neurons innervating the corpus cavernosum, and eNOS protein expression has

been identified primarily in both cavernosal smooth muscle and endothelium [7]. In mice, nNOS has been located to the dorsal penile nerve and its branches in the mouse penis [8] as well as in intrinsic nerves of the erectile tissue [20]. Also, it has been reported that eNOS has been located in mouse erectile tissue, and an up regulation of eNOS has been suggested to compensate for loss of the neuronal isoform in nNOS transgenic mice with normal erectile responses [8]. Neurogenic NO is still considered the most important factor responsible for immediate relaxation of penile vessels and the corpus cavernosum. However, endothelium-generated NO seems essential for maintaining erection [1]. Impairment of the mechanisms that support the relaxation of corpus cavernosum smooth muscle may lead to impotence [34, 38]. Erectile dysfunction is defined as the consistent inability to achieve or maintain an erection sufficient for satisfactory sexual performance [29]. Studies have shown that erectile dysfunction is caused by inadequate relaxation of the corpus cavernosum with defects in NO production [4, 6]. Clinical and experimental studies have also demonstrated that chronic ethanol treatment interferes with either NO production or the release of NO from endothelial cells [35, 36, 40]. However, there are contradictory reports about the influence of chronic ethanol on vascular responsiveness. Chronic ethanol consumption has been shown to either decrease [35] or increase [19, 27] the release of NO from endothelial cells. On the other hand, Williams et al. [44] has shown that chronic ethanol treatment did not affect the relaxation produced by acetylcholine, ATP or sodium nitroprusside in aorta with or without endothelium. Although the effects of chronic ethanol have been widely studied in vascular preparation – aorta, thoracic aorta, and mesenteric artery – there is a little evidence about the influence of ethanol on NO production and/or its release in corpus cavernosal tissue. Saito et al. [39] reported that chronic ethanol treatment significantly enhanced relaxations of rabbit corpus cavernosum to EFS and bethanechol. However, to our knowledge, the effect of ethanol treatment on the endothelium of corpus cavernosum in mice has not been studied functionally or histologically. Therefore, in the present study, the *in vitro* responses to electrical field stimulation, acetylcholine and exogenous NO were characterized in the isolated corpus cavernosal tissue preparations from subacute ethanol-treated mice. In addition, we examined whether the ethanol treatment induced degenerative changes selectively in the endothelial cells of corpus cavernosum from ethanol-treated mice using electron microscopy.

Materials and Methods

Animals

Male Swiss albino mice (20–25 g) were obtained from Cukurova University Experimental Research Center (TIBDAM). The mice were housed in Plexiglas cages and kept in a temperature- and light-controlled environment (light/darkness cycle: 12-h light/dark cycle) and allowed free access to food and water.

The local Ethics Committee of the University of Cukurova approved all experiments.

Ethanol treatment

For subacute ethanol administration, we used a method described by Goldstein [17]. Mice were put in an inhalation chamber and continuously exposed to ethanol vapor by inhalation for 7 or 14 days. The inhalation chamber consisted of a Plexiglas box (7.5 l) through which ethanol vapor continuously passed. 95% Ethanol was delivered to filter paper at a rate of 0.0035 ml/min by a peristaltic pump. The control group was not exposed to ethanol vapor but only placed in air vapor chambers under conditions similar to those of the ethanol group. At the end of the 7 or 14 days of ethanol inhalation, control and inhalated mice were removed from the chamber, and under anesthesia with halothane (2%), the thorax was opened and cardiac blood was immediately taken by Pasteur pipette and collected in NaF containing tubes. Then, the blood ethanol level was measured by headspace gas chromatography.

Drugs and solutions

The following drugs were used (supplied by Sigma Chemical Co., St. Louis, MO, USA, unless stated otherwise): acetylcholine chloride, L-arginine hydrochloride, N^{ω}-nitro-L-arginine, 1*H*-[1,2,4]oxadiazolol-[4,3-*a*]quinoxalin-1-one (ODQ), papaverine, phenylephrine hydrochloride, ethanol (SISMED Medical Limited Co.), and sodium nitrite (Merck, Darmstadt, Germany). Acidified sodium nitrite, which was used

as an exogenous NO source, was prepared daily for each experiment by diluting sodium nitrite in deaerated water acidified to pH 2.0 with HCl [9]. All drugs were dissolved in distilled water except ODQ, which was dissolved in dimethylsulphoxide; the final concentration of this solvent was shown to have no biological effects.

Functional experiments

Tissue preparations

Control and ethanol-treated mice were killed by cervical dislocation. Penises from control and ethanol-treated mice were removed and 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, $NaPO_4$ 1.2, glucose 11). The glans penis and urethra were excised and the fibrous septum between two corpus cavernosum strips were cut and each corpus cavernosum was carefully dissected from the adherent tissues, keeping the tunica albuginea intact. Cavernosal strips were mounted under 0.2 g of tension in an organ bath (15 ml) containing Krebs solution. The bath medium was maintained at 37°C and gassed with 5% CO_2 and 95% O_2. The tissue strips were allowed to equilibrate for a period of 60 min. During equilibration, the bath solution was replaced every 15 min. The responses were recorded with an isotonic transducer (Ugo Basile, 7006) on a recorder (Ugo Basile Gemini, 7070).

Experimental protocol

To investigate the effects of ethanol treatment on the nitrergic relaxation, electrical field stimulation, exogenous NO and acetylcholine were studied as follows. Following 60 min of equilibration, cavernosal strips from control and ethanol-treated mice were pre-contracted with 5×10^{-6} M phenylephrine. After a steady state of contraction was obtained, to study relaxant action, electrical field stimulation (EFS; 8 Hz, 30 V, 0.5 ms, 15 s-train; Grass S88 stimulator), exogenous NO (10^{-4} M; administered as acidified $NaNO_2$) and acetylcholine (10^{-7} M) were applied at 3–5 min intervals without rinsing the tissue between each individual application to a single tissue. Thus, the first series of relaxant responses was obtained. After a 30-min washout period, the second series of responses were recorded in the same manner. At the end of the experimental protocol, papaverine (10^{-4} M) was added to the organ bath to achieve maximal relaxation. Also, the frequency of electrical field stimulation and concentration of exogenous NO and acetylcholine were chosen according to previous studies in mouse corpus cavernosum in our laboratory [14–16]. In some experiments, the effects of the nitric oxide synthase inhibitor N^{ω}-nitro-L-arginine (10^{-4} M) or selective soluble guanylate cyclase inhibitor ODQ (10^{-4} M) were investigated on relaxations in response to EFS, exogenous NO and acetylcholine. In control and 14 day ethanol-treated mice, after the first series of relaxant responses were recorded, the tissue was incubated in a medium containing N^{ω}-nitro-L-arginine (10^{-4} M) or selective soluble guanylate cyclase inhibitor 1*H*-[1,2,4]-oxadiazolol[4,3-*a*]quinoxalin-1-one (ODQ; 10^{-4} M), and at the end of 30 min of incubation, the effects of a second series of EFS, exogenous NO and acetylcholine was examined.

Histological study

For transmission electron microscopy (TEM), corpus cavernosum strips isolated from 14 day ethanol-treated and control mice were fixed in 2.5% glutaraldehyde for 4 h. After fixation, the specimens were rinsed in 0.1 M phosphate buffer, pH 7.2, postfixed in 2% OsO_4 in 0.1 M phosphate buffer for 2 h, and then rinsed in distilled water, dehydrated in a graded ethanol series and put into propylene oxide twice. Following this, they were embedded in epoxy resin and left at 70°C for 12 h to complete polymerization. With a Leica UCT 125® Ultramicrotome (Leica Microsystems GmbH, Wien, Austria), 1 μm semi thin sections were cut and stained with toluidine blue. These sections were observed with a light microscope to determine appropriate areas for ultra thin sections. Then, 70 nm ultra thin sections were obtained and stained with uranyl acetate and lead nitrate. The photographs were taken with a Megaview III® Digital camera (Olympus® Soft Imaging Solutions GmbH, Münster, Germany) attached to a JEOL® JEM-1011 (Jeol Ltd. Tokyo, Japan) Electron Microscope.

Statistical analysis

The relaxation of mouse cavernosal strips produced by electrical field stimulation, exogenous NO and acetylcholine was calculated as the percentage of re-

laxation induced by papaverine at the end of the experiment. Results were expressed as the means ± SEM, and *n* refers to the number of animals used for each experiment. For statistical analysis, one way analysis of variance (ANOVA) was used, followed by the Bonferroni post hoc test or Student's *t*-test. p values less then 0.05 were considered to be significant.

Results

Blood ethanol level in control and ethanol-treated mice

The blood ethanol levels were 110.4 ± 13 mg/dl and 114.8 ± 11 mg/dl in 7 and 14 day ethanol-treated mice (95%; 0.0035 ml/min), respectively, whereas it was 5.3 ± 12.8 mg/dl in control mice (n = 8; p > 0.05). There was no significant difference in ethanol level between 7 and 14 day ethanol-treated mice for (p > 0.05; data not shown).

Effects of EFS, NO and acetylcholine on the corpus cavernosum from ethanol-treated mice

Electrical field stimulation (EFS; 8 Hz, 30 V, 0.5 ms, 15 s-train), NO (10^{-4} M) and acetylcholine (10^{-7} M) induced reproducible relaxation of the corpus cavernosum obtained from control as well as 7 day ethanol treated-mice. The EFS, NO and acetylcholine-induced relaxations were 27.1 ± 2.9, 35.4 ± 6.5 and 33.2 ± 5.1% in the first series of responses and 35.9 ± 2.9, 40.4 ± 5.7 and 38.8 ± 6% in the second, respectively, when calculated as a percentage of papeverine (10^{-4} M) in control mice for 7 days (Fig. 1A). Similar responses were observed on corpus cavernosum strips of 7 day ethanol-treated mice. The nitrergic relaxations induced by the EFS, NO and acetylcholine were 33.2 ± 5.2, 29.3 ± 4.8 and 37.1 ± 7.7%, respectively, in the first series of responses and 39.6 ± 5.3, 37.3 ± 7.5 and 42.6 ± 7.5% in the second series in 7 day ethanol-treated mice (Fig. 1B). There was no significant difference in nitrergic relaxations between the control and 7 day ethanol-treated mice (p > 0.05; n = 12; Fig. 1C).

We also studied the influence of ethanol treatment for 14 days on the nitrergic relaxation of corpus cavernosum of mice. In control groups for 14 days, the amplitudes of EFS, NO (10^{-4} M) and acetylcholine (10^{-7} M)-induced relaxations were 30.2 ± 3.2, 39.1 ± 5.5 and 31.7 ± 4.1% in the first series of responses

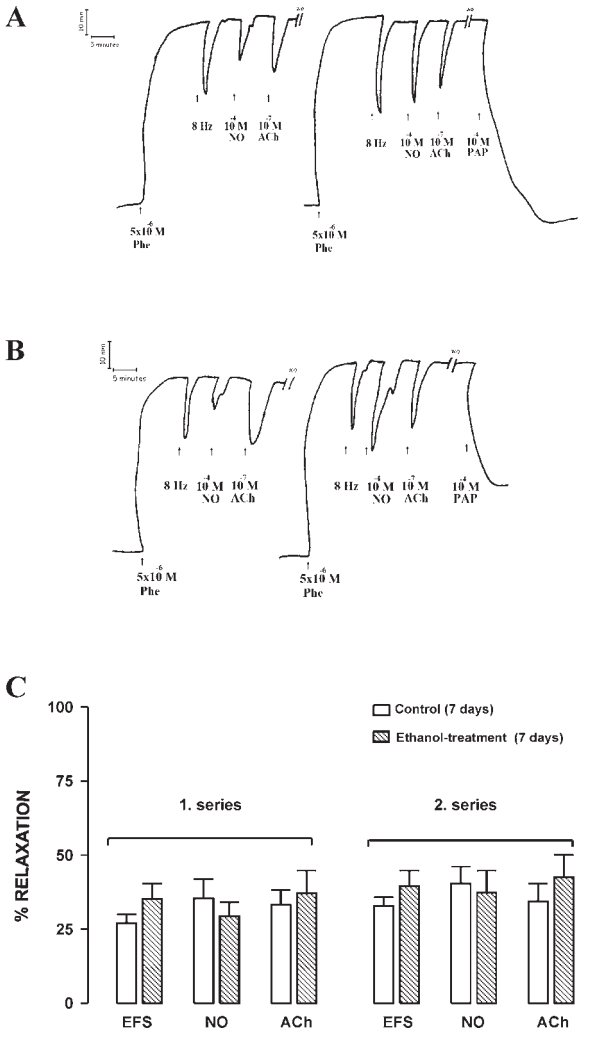

Fig. 1. Representative traces showing the relaxation of control **(A)** and 7 day ethanol-treated **(B)** corpus cavernosal strips of mice in response to electrical field stimulation (EFS; 8 Hz, 30 V, 0.5 ms, 15 s-train), exogenous nitric oxide (NO; 10^{-4} M) and acetylcholine (ACh; 10^{-7} M). Bar graph (C) showing the effect of ethanol treatment for 7 days on the relaxation to EFS, NO and ACh in mouse corpus cavernosal strips pre-contracted with 5 × 10^{-6} M phenylephrine

and 33.7 ± 2.6, 41.2 ± 4.6, and 33.3 ± 4.9% in the second series, respectively (Fig. 2A). On the other hand, relaxant responses of corpus cavernosum to EFS and NO did not change while relaxation to acetylcholine was significantly diminished in 14 day ethanol-treated mice compared to the control group (p < 0.05; n = 17; Fig. 2B and C). The nitrergic relaxations induced by the EFS, NO and acetylcholine were 27.8 ± 4.2, 42.7 ± 5.4 and 10.2 ± 2.1% in the first series of responses and 30.26 ± 3.7, 46.66 ± 5.9 and 9.25 ± 3.0% in the second series, respectively, in 14 day ethanol-treated mice.

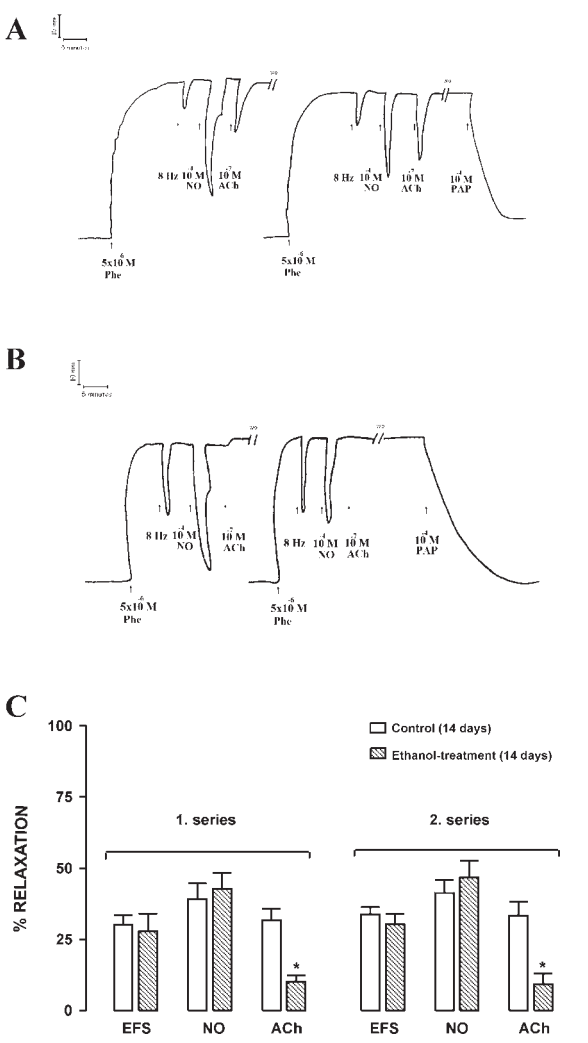

Fig. 2. Representative traces showing the relaxation of control **(A)** and 14 day ethanol-treated **(B)** corpus cavernosal strips of mice in response to electrical field stimulation (EFS; 8 Hz, 30 V, 0.5 ms, 15 s-train), exogenous nitric oxide (NO; 10^{-4} M) and acetylcholine (ACh; 10^{-7} M). Bar graph **(C)** showing the effect of ethanol treatment for 14 days on the relaxation to EFS, NO and ACh in mouse corpus cavernosal strips pre-contracted with 5 × 10^{-6} M phenylephrine. All values are the means ± SEM. * $p < 0.05$ significantly different from control, one-way ANOVA followed by the Bonferroni multiple comparison *t* test

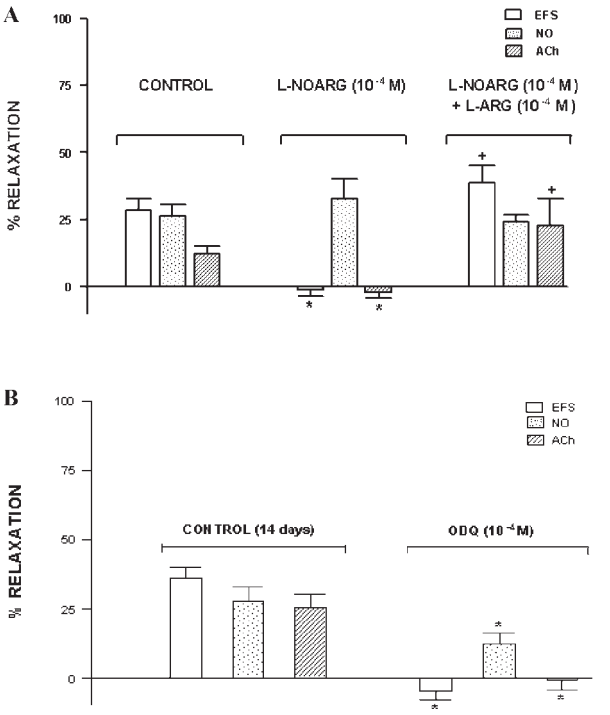

Fig. 3. Effects of L-nitroarginine (L-NOARG; 10^{-4} M), L-NOARG plus L-arginine (L-ARG; 10^{-4} M) **(A)** and ODQ (10^{-4} M) **(B)** on the relaxant responses to electrical field stimulation (EFS; 8 Hz, 30 V, 0.5 ms, 15 s-train), exogenous nitric oxide (NO; 10^{-4} M) and acetylcholine (ACh; 10^{-7} M) in corpus cavernosal strips of control mice. All values are the means ± SEM. * $p < 0.05$ significantly different from control; $^{+}$ $p < 0.05$ significantly different from L-NOARG, one-way ANOVA followed by the Bonferroni multiple comparison *t* test

Effect of N$^{\omega}$-nitro-L-arginine on relaxations to EFS, NO and acetylcholine relaxation in ethanol-treated mice for 14 days

In both the control and 14 day ethanol-treated mice, treatment of the mouse corpus cavernosum with N$^{\omega}$-nitro-L-arginine (10^{-4} M) abolished the relaxations to EFS (8 Hz, 30 V, 0.5 ms, 15 s-train) and acetylcholine (10^{-7} M) ($p < 0.05$; n = 8; Fig. 3A and 4) but not those to exogenous NO (10^{-4} M). The inhibitory effect of N$^{\omega}$-nitro-L-arginine on the relaxant responses of corpus cavernosum to EFS (–10.1 ± 5.9%; n = 8) and acetylcholine (10^{-7} M; –5.1 ± 5.4%; n = 8) in 14 day ethanol-treated mice was not significantly different from the response to electrical field stimulation (–4.33 ± 2.2%; n = 8) and acetylcholine (–2 ± 2.3%; n = 8) in the control group ($p > 0.05$). The inhibitory effect of N$^{\omega}$-nitro-L-arginine on electrical field stimulation and acetylcholine was prevented by pre-incubation with L-arginine (10^{-4} M) for 30 min in both the control and ethanol-treated groups ($p < 0.05$; n = 8; Fig. 3A and 4B).

Effect of ODQ on relaxations to EFS, NO and acetylcholine relaxation in 14 day ethanol-treated mice

Treatment of the corpus cavernosum strips obtained from control and 14 day ethanol-treated mice with ODQ (10^{-4} M) completely inhibited the relaxation to

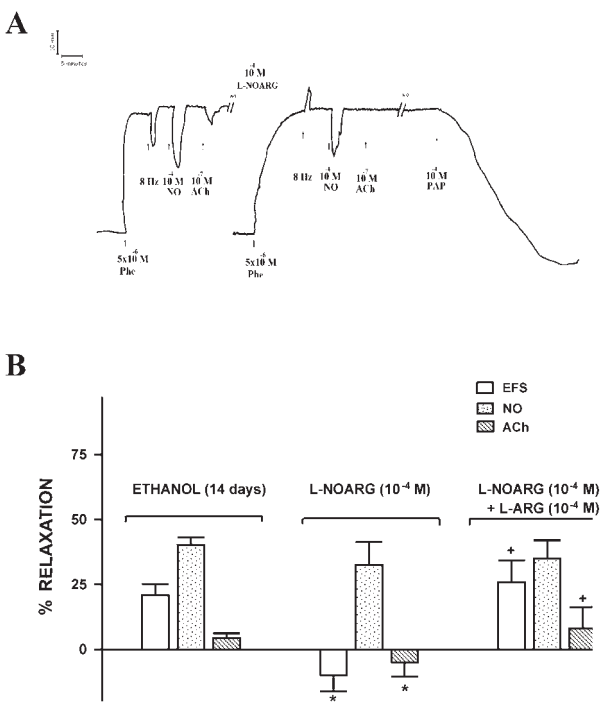

Fig. 4. Representative trace **(A)** and bar graph **(B)** showing the effect of L-nitroarginine (L-NOARG; 10^{-4} M) and L-NOARG plus L-arginine (L-ARG; 10^{-4} M) on the relaxant responses to electrical field stimulation (EFS; 8 Hz, 30 V, 0.5 ms, 15 s-train), exogenous nitric oxide (NO; 10^{-4} M) and acetylcholine (ACh; 10^{-7} M) in corpus cavernosal strips of 14 day ethanol-treated mice. All values are the means ± SEM. * $p < 0.05$ significantly different from control; $^{+}$ $p < 0.05$ significantly different from L-NOARG, one-way ANOVA followed by the Bonferroni multiple comparison *t* test

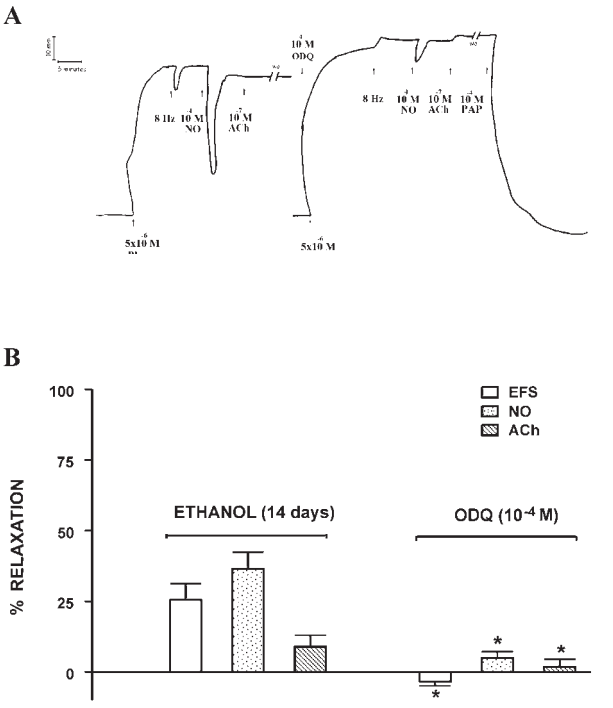

Fig. 5. Representative trace **(A)** and bar graph **(B)** showing the effect of ODQ (10^{-4} M) on the relaxant responses to electrical field stimulation (EFS; 8 Hz, 30 V, 0.5 ms, 15 s-train), exogenous nitric oxide (NO; 10^{-4} M) and acetylcholine (ACh; 10^{-7} M) in corpus cavernosal strips of 14 day ethanol-treated mice. All values are the means ± SEM. * $p < 0.05$ significantly different from control, one-way ANOVA followed by the Bonferroni multiple comparison *t* test

EFS, NO (10^{-4} M) and acetylcholine (10^{-7} M), and the relaxation induced by EFS and acetylcholine returned to contraction in the presence of ODQ ($p < 0.05$; n = 8; Fig. 3A and 5). The inhibitory effect of ODQ on the relaxant responses of corpus cavernosum to EFS, NO (10^{-4} M; 4.9 ± 2.3) and acetylcholine (10^{-7} M; 1.8 ± 2.7%; n = 8) in 14 day ethanol-treated mice was not significantly different from the response to electrical field stimulation (–4.6 ± 3.1%; n = 8), exogenous NO (10^{-4} M; 7.6 ± 3.6%) and acetylcholine (–1 ± 3.4%; n = 8) in the control group ($p > 0.05$).

Electron microscopy evaluation

Endothelial cells isolated from the corpus cavernosum of the control group and 7-day ethanol-treated group were observed as quite healthy with euchromatic nuclei, evident nucleoli and thin cytoplasms (Fig. 6A). Besides mitochondria, many pinocytotic vesicles were noticed in the cytoplasm**,** and the junctional complexes and basal lamina were also evaluated as normal. On the other hand, major changes were observed in nuclei of endothelial cells of corpus cavernosum from 14 day ethanol-treated mice (Fig. 6B). Evident heterochromatin was seen in the nuclei. Moreover, this heterochromatin was condensed and located peripherally. In addition, deep nuclear indentations were obvious. A decrease in the number of pinocytotic vesicles was determined in many endothelial cells**.** There were vacuolization in the cytoplasm of some endothelial cells. The junctional complexes between the endothelial cells, basal lamina and smooth muscle cells did not show any alterations.

Discussion

In the present study, endothelial damage in corpus cavernosum treated with ethanol for 14 days was

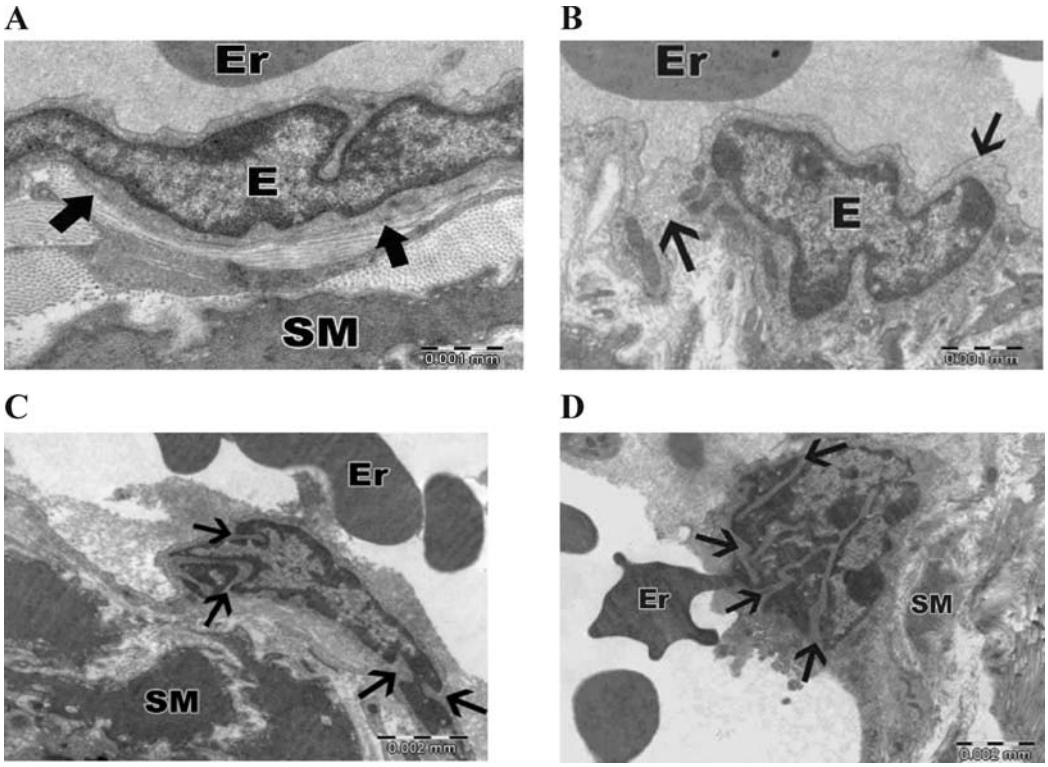

Fig. 6. Electron photomicrographs of endothelial cells in the sinus of corpus cavernosa of penis in control **(A, B)** and 14 day ethanol-treated mouse **(C, D)** strips. **(A, B)** Endothelial cells (E) are clearly seen as quite healthy with euchromatic nuclei, mitochondria, and pinocytotic vesicles in the cytoplasm. **(C, D)** Cell size is reduced compared to (A, B) and the organelles seen in (A, B) are hardly seen in the cytoplasm. The cytoplasmic processes are dramatically shrunken (arrows). SM – smooth muscle; Er – erythrocyte

shown both by histological studies and by the altered relaxant response to the endothelium-dependent vasodilator acetylcholine in the corpus cavernosa of mice. Ethanol treatment also induced degeneration of the endothelial cells in the corpus cavernosa of the penis. Endothelium-dependent relaxation induced by acetylcholine significantly diminished, whereas the relaxant responses to electrical field stimulation and exogenous NO were unimpaired in ethanol-treated strips and was not different from control responses, suggesting that treatment with ethanol under the conditions used in this study is considered to impair the endothelial function.

Penile erection is dependent upon cavernous smooth muscle relaxation, and it has been shown that NO, released from nerves, is the major relaxant agent in several mammals [2]. This has also been suggested for isolated mouse erectile tissues [8, 14, 20, 28]. Additionally, nNOS has been observed in the dorsal penile nerve and its branches in the mouse penis [8, 28]. The present study verified that pre-contracted preparations of mouse corpus cavernosum respond with relaxations to electrical field stimulation of nerves under the experimental conditions, and showed that the relaxations were significantly inhibited by blockade of the synthesis of NO and soluble guanylate cyclase with L-nitroarginine (L-NOARG) and ODQ, respectively. These findings support the hypothesis that NO synthesized from L-arginine mainly mediates the relaxation of penile corpus cavernosum muscle to nerve stimulation in mouse. Gocmen et al. [14] showed that EFS (10 Hz)-induced relaxation of mouse corpus cavernosum strips is completely inhibited by tetrodotoxin, suggesting this response is neurogenic. In addition, it has been shown that endothelium-derived NO released from vascular endothelium, which lines the sinusoidal spaces of the corpus cavernosum, is involved in the relaxation of the cavernous smooth muscle [2, 23, 32]. Endothelial NOS (eNOS) has previously been found in the mouse erectile tissue, and an up-regulation of eNOS has been suggested to compensate for loss of the neuronal isoform in nNOS transgenic mice with normal erectile responses [8]. Acetylcholine-induced relaxation of erectile tissue

has been shown to involve the release of NO from endothelial cells [2]. Confirming the findings in mice [14, 28], endothelium-dependent relaxations induced by acetylcholine were significantly attenuated by L-NOARG and ODQ in the erectile tissue from control mice in the present study, and inhibition induced by L-NOARG was prevented by the addition of L-arginine, suggesting that NO from the endothelium is involved in the response. Similar findings have been observed in isolated rabbit [26], canine [30], and human [25] corpus cavernosa strips.

Also, to our knowledge, the effect of ethanol treatment on the endothelium of corpus cavernosum in mice has not been studied. In the present study, we investigated the effects of ethanol treatment on the nitrergic relaxation of the neuronal and endothelial source in mice corpus cavernosum. In the various studies, the inhalation technique has been used for ethanol treatment [3, 12, 17, 31]. For ethanol administration in the present study, we used the ethanol inhalation technique described by Goldstein [17]. We found that blood ethanol levels were 110.4 ± 13 mg/dl and 114.8 ± 11 mg/dl in 7 and 14 day ethanol-treated mice, respectively, and there was no significantly difference in ethanol levels between 7 and 14 day ethanol-treated mice. These results are consistent with those of Tirapelli et al. [43], who also found that the blood ethanol levels were not significantly different among the 2-, 6- and 10-week periods of treatment. In the present study, histological studies demonstrate that ethanol treatment induced degenerative changes selectively in the endothelial cells of corpus cavernosum from 14 day ethanol-treated mice but these were not observed in the endothelial cells of 7 day ethanol-treated mice. The reason for this difference is not entirely clear, but a contributing factor may be the duration of ethanol treatment.

In the present study, treatment with ethanol selectively impaired the endothelial function. Multiple deep and shallow nuclear indentations and irregularity of the nuclear form of the endothelial cells provided an increased contact area between the nucleus and cytoplasm. In most cases, this seems to demonstrate increased nucleocytoplasmic exchange and metabolic activity [13]. In our study, it is thought that increased metabolic activity may be due to the cellular healing process in response to ethanol related endothelial damage. In contrast to endothelium cells, such degeneration is not observed in the smooth muscle cells.

In the functional experiments, ethanol treatment for 14 days abolished the endothelium-dependent relaxations induced by acetylcholine but failed to alter the relaxation in response to EFS and exogenous NO. Despite the histological and functional damage caused to endothelial cells by ethanol, the nerve and smooth muscle seem to be functionally unaffected, and the inhibitory effect of ethanol treatment on acetylcholine-induced relaxation has been correlated with histological findings. Therefore, treatment with ethanol under the conditions used in this study is considered to selectively impair the endothelial function. In contrast to our findings, Saito et al. [39] reported that ethanol treatment significantly enhanced the relaxations in response to EFS and bethanechol in the rabbit corpus cavernosum. Similarly, Hatake et al. [19] showed that the endothelium-dependent relaxation responses to acetylcholine and calcium ionophore A23187 were greater in ethanol-fed rat aortic strips than in the control. On the other hand, in the rat thoracic aorta, ethanol significantly reduced the aortic relaxation to acetylcholine [22]. In contrast, Williams et al. [44] demonstrated that ethanol treatment did not affect the relaxation produced by acetylcholine in rat thoracic aorta. There are contradictory reports about the influence of ethanol treatment on endothelium-dependent relaxation. These conflicting reports may be related to differences in dose and/or duration of ethanol administration or different experimental protocols.

Clinical and experimental studies have also demonstrated that ethanol treatment interferes with either NO production or the release of NO from endothelial cells [35, 36, 40]. In our ethanol-treated group, endothelial cells of the corpus cavernosum appear to be altered, and acetylcholine-induced endothelium-dependent relaxation is inhibited with respect to the control, suggesting that ethanol treatment decreases NO release from the endothelium *via* an endothelial impairment. The decreased endothelial NO availability may either be related to reaction with superoxide anion to form peroxynitrite radicals [5] or the oxidative inactivation of endothelial NOS by ethanol-induced free radicals [21, 33]. Also, recent studies of vascular responses in rat pial arterioles in animals chronically fed alcohol [42] suggest that alcohol impairment of the arteriolar vasodilatory response to acetylcholine can be reversed by treatment with superoxide dismutase, providing evidence that impaired vasodilatation may be due to enhanced vascular release of oxygen-derived free radicals. Furthermore, Grattaglione et al.

[18] reported that increased xanthine oxidase activity and decreased levels of glutathione may be responsible for enhanced free-radical production and lack of protection against oxidative stress in chronic alcoholic patients. Another possibility to explain alcohol-induced endothelial damage is related to modifications in the lipoprotein profile because impairment of endothelium-dependent vasodilatation was also demonstrated in hypercholesterolemic patients [10]. In chronic alcoholic patients, alcohol has been shown responsible for an increase in the oxidizability of LDL and decrease in HDL levels [11]. Such effects of alcohol may indirectly induce endothelial dysfunction as well as predispose an individual to early pathogenesis of the atherosclerotic lesion. The role of alcohol as a direct cytotoxic agent on endothelium has been addressed in previous studies [24, 45]. In our group of ethanol-treated mice, smooth muscle cells were not altered histologically, and endothelium-independent relaxations induced by EFS and exogenous NO and inhibition of these responses with L-NOARG and ODQ were similar with respect to controls, suggesting that ethanol had no cytotoxic effect on the tissue or the NOS-cyclic GMP signal transduction system.

The data of this study further show that chronic ethanol treatment did not significantly change corpus cavernosal contraction induced by phenylephrine, a selective α_1-adrenergic agonist, as compared to the control group. Similar findings have been observed in rat thoracic aorta [22, 44]. In contrast, both an enhanced [35] and an attenuated [41] response to phenylephrine-induced contraction in rat thoracic aorta has been shown in response to treatment with ethanol. These differences may be related to differences in dose and/or duration of ethanol administration or different experimental protocols.

In conclusion, the present study histologically showed that ethanol treatment caused damage to endothelial cells but not smooth muscle cells. Also, it demonstrated that endothelium-dependent relaxation induced by acetylcholine significantly diminished while the relaxant responses to EFS and exogenous NO were not altered in strips of corpus cavernosum from ethanol-treated mice. This suggests that treatment with ethanol under the conditions used in this study impairs the endothelial function, and that ethanol treatment may lead to erectile dysfunction through reduced NO release.

Acknowledgment:
This work was supported by the Cukurova University Research Foundation (TF.2005YL5).

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Received:
January 3, 2008; in revised form: September 4, 2008.