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ORIGINAL ARTICLE

CHANGES IN THE NEUROGENIC AND ENDOTHELIUM-DEPENDENT RELAXANT RESPONSES OF RABBIT CORPUS CAVERNOSUM SMOOTH MUSCLE AFTER CAVERNOUS NERVE NEUROTOMY

T. Utkan[1], Y. Sarioglu[2] and Y. Yazir[3]

[1]Department of Pharmacology, Faculty of Medicine and Experimental Medical Research Center, Kocaeli University, 41380, Kocaeli-Turkey; [2]Department of Pharmacology, Faculty of Medicine, Gazi University, 06500, Ankara-Turkey; [3]Department of Histology and Embryology, Faculty of Medicine, Kocaeli University, 41380, Kocaeli-Turkey

SUMMARY

The present study was conducted to investigate the reactivity of the corpus cavernosum smooth muscle after unilateral cavernous nerve neurotomy in rabbits. Rabbits (18) were randomly divided into two groups: sham-operated (n = 9) and those subjected to unilateral neurotomy of a 5-mm segment of the cavernous nerve (n = 9). The reactivity of the corpus cavernosum tissue from the neurotomized and sham groups was studied in organ chambers at 4 weeks postoperation. In the neurotomized group, endothelium-dependent relaxation of the corpus cavernosum smooth muscle to carbachol was significantly increased when compared to the sham group. In addition, the sensitivity (i.e., pD_2) of neurotomized strips to carbachol was also increased when compared to controls. Electrical field stimulation-induced neurogenic relaxation was significantly reduced in the neurotomized group. Relaxation to the nitric oxide (NO) donor sodium nitroprusside and to papaverine was similar in the cavernosal tissue of both groups. There was no change in agonist potency. Furthermore, neurotomy had no effect on KCl-induced contractile responses. When tissue contraction was induced with phenylephrine to study relaxation to various stimuli, the tension induced was similar in the neurotomized and the sham control groups. We conclude that unilateral, chronic cavernous nerve neurotomy causes significant functional changes to the penile erectile tissue of rabbits, which may contribute to the development of impotence.

INTRODUCTION

Penile erection is related to the increased penile arterial flow, sinusoidal relaxation, venous outflow and sufficient autonomic innervation of the corpus cavernosum, as well as normal functioning of the smooth muscle cells (1, 2). A defect in any of these systems may result in impotence. There are strong associations between several neurological diseases and erectile dysfunction (3). The cavernous nerves run between the major pelvic ganglion and the penile corpus cavernosum and spongiosum (4). Erectile dysfunction in men after iatrogenic damage of the cavernous nerve during surgery has been previously reported, these data confirming that the cavernous nerve plays a major role in the regulation of penile erection (5, 6).

Correspondence: Tijen Utkan, Prof. Dr., Kocaeli University, Faculty of Medicine, Department of Pharmacology and Experimental Medical Research Center, 41380 Umuttepe Kocaeli-Turkey. E-mail: tijenutkan@gmail.com.

Relaxation of trabecular smooth muscle is needed to achieve and maintain penile erection (7). Nitric oxide (NO) which is released by nitrergic nerves in the trabecular and penile arterial tissues, as well as the endothelium that lines the lacunar spaces and the intima of penile arteries, is a key mediator of penile smooth muscle relaxation. NO activates guanylate cyclase, leading to the elevation of intracellular levels of cGMP and penile smooth muscle relaxation (8, 9). Also, previous in vitro studies have indicated that corporal smooth muscle relaxation may be impaired in a large proportion of impotent men (10, 11), as well as in experimental animal models (12-14).

The purpose of this study was therefore to determine the effects at 4 weeks after unilateral cavernous nerve neurotomy on the NO/cGMP-mediated relaxant responses of penile corporal smooth muscle. In addition, we also evaluated the histopathological changes in the neurotomized rabbits using light microscopy.

MATERIALS AND METHODS

Animals

Eighteen sexually mature male albino white rabbits weighing 2.5-3.0 kg were used. All experiments were conducted according to the rules of the Animals Ethics Committee of the Kocaeli University Faculty of Medicine. After the animals were anesthetized with pentobarbital (60 mg/kg i.p.) a midline incision was made from the umbilicus to the pubis. The urinary bladder was retracted laterally to locate the major pelvic plexus on the lateral surface of the prostate. In the sham-operated control group (n = 9) the right cavernous nerve was identified but not divided at the time of operation. In the cavernous nerve-neurotomized group (n = 9) a 5-mm segment of the right cavernous nerve running caudally from the pelvic plexus was unilaterally surgically excised. The abdominal wall and the skin were closed in two layers with a 3-0 silk suture. Four weeks later the following studies were carried out. The animals were sacrificed with an overdose of pentobarbital and the penises were excised. The tunica albuginea was cleared of overlying tissues and opened. The proximal half of the corporal body was dissected free from the tunica and harvested en block, as previously described (12-14). In neurotomized and control animals, the right cavernosal tissue was used for isometric tension measurement in an organ bath and macroscopically evaluated. The specimens were fixed in 10% neutral buffered formalin solution for 72 h, and serial transverse 5 µm-thick sections from paraffin blocks were stained with hematoxylin and eosin. A blind observation and assessment of all sections was done microscopically.

Organ bath studies

Isometric tension of the corpus cavernosum smooth muscle was studied as previously described (12-14). Briefly, strips of corpus cavernosum tissue measuring approximately 2 x 2 x 15 mm in a 20-mL water-jacketed tissue bath with physiological salt solution were assayed for isometric tension measurement. The strips were tied with silk to a force transducer (Grass FT03, Quincy, MA, USA) at one end and fixed with silk ties to a glass support at the other end. The transducer output was recorded on a Grass polygraph model 79E. The solution was gassed with 95% O_2 and 5% CO_2 during the study and the temperature was maintained at 37 °C by a thermoregulated water circuit. Resting load was set at 2 g, a value that was previously found to be optimal for the measurement of changes in the tension of rabbit corpus cavernosal preparations (14). The preparations were allowed to equilibrate in Krebs–bicarbonate for 1 h, during which time the Krebs–bicarbonate was refreshed every 15 min. After equilibration, the strips were contracted with phenylephrine (10 µM). This concentration produced 70-80% of the maximal response to phenylephrine. Relaxations to pharmacological treatment and to transmural electrical field stimulation (EFS) were studied after the phenylephrine-induced contraction had reached a plateau level. Concentration–response relationships for carbachol (10 nM-100 µM), sodium nitroprusside (10 nM-100 µM) and papaverine (1-100 µM) were obtained by adding one of those agents to the bath in a cumulative manner. Drugs were dissolved so that for every concentration the volume added to the chamber was 100 µL. EFS was provided by a stimulator (Grass S 88) and applied via two platinum wire electrodes set vertically at the opposite sides of the organ bath with the suspended tissue. Prior to electrical stimulation, tissue was treated with guanethidine (5 µM) (adrenergic nerve blocker) and atropine (1 µM) (muscarinic receptor blocker) for 30 min. Square-wave pulses of 10 V at 0.5-ms duration, in 10-s trains with various frequency (2-32 Hz) were applied at 5-min intervals. For each frequency the strips were allowed to return to the baseline precontractile tension between tests.

Drugs

The ionic composition of the Krebs–bicarbonate solution was as follows (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11 (14). Fresh solutions were prepared on the day of the experiments. The following drugs were all obtained from Sigma Chemical Co. (St. Louis, MO, USA): carbachol chloride, phenylephrine hydrochloride, sodium nitroprusside, atropine sulfate, guanethidine sulfate, papaverine hydrochloride. All drugs were dissolved in distilled water and freshly prepared on the day of the experiment.

Statistical analysis

Experimental values were expressed as the mean ± SEM. The relaxant effect of agonists was expressed as a percentage of the precontraction to phenylephrine. To evaluate the effects of agonist, maximum responses (E_{max}) and pD_2 values (i.e., the negative logarithm of the concentration for the half-maximal response; $-\log EC_{50}$) were calculated. Statistical comparisons between groups were performed using the unpaired Student's *t*-test. Probabilities of less than 5% ($P < 0.05$) were considered significant.

RESULTS

Using light microscopy, there were no significant differences between the groups in the corpus cavernosum and vascular systems (Fig. 1).

Carbachol (10 nM-100 µM), sodium nitroprusside (10 nM-100 µM) and papaverine (1-100 µM) produced concentration-dependent relaxation in submaximally (70-80% of maximal contraction) precontracted (10 µM phenylephrine) corpus cavernosum strips obtained from sham-operated and neurotomized groups. Where tissues were contracted with phenylephrine to assess responses to relaxant agonists, tension induced was similar in the two groups. The tension was 1231 ± 141 mg and 1283 ± 71.2 mg (mean ± S.E) in the neurotomized and sham control groups, respectively. Endothelium-dependent relaxation to carbachol was significantly increased in the neurotomized group when compared to the sham-operated group ($P < 0.05$). The concentration–response curve for carbachol was shifted to the left with significantly higher pD_2 values ($P < 0.05$) (Fig. 2, Table I). Relaxation of cavernosal tissue to the NO donor sodium nitroprusside in the neurotomized group was similar to the sham-operated group and there were no significant changes in the pD_2 values. The relaxant responses of corporal strips from the neurotomized group to papaverine (1-100 µM) were not significantly different from those obtained with corporal strips from sham-operated rabbits (Table I).

In precontracted strips, electrical stimulations (2-32 Hz) evoked frequency-dependent relaxations. The EFS-induced relaxation in the

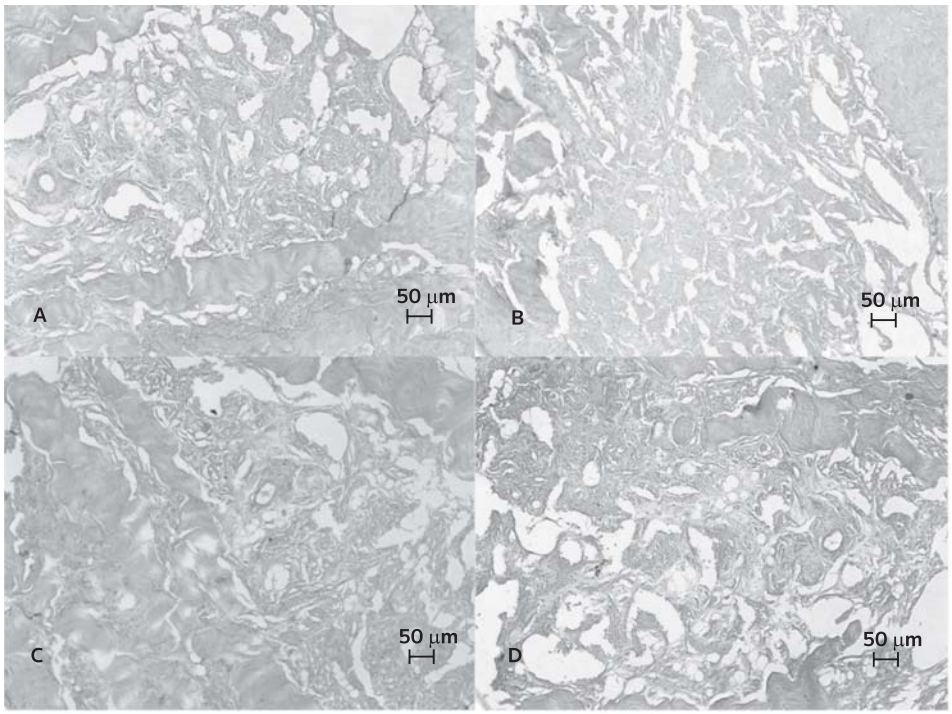


Figure 1. Histological sections of the rabbit corpus cavernosum isolated from sham-operated (**A**. right side, **B**. left side) and 4-week unilateral cavernous nerve neurotomy group (**C**. right side, **D**. left side) (H+E X 100).

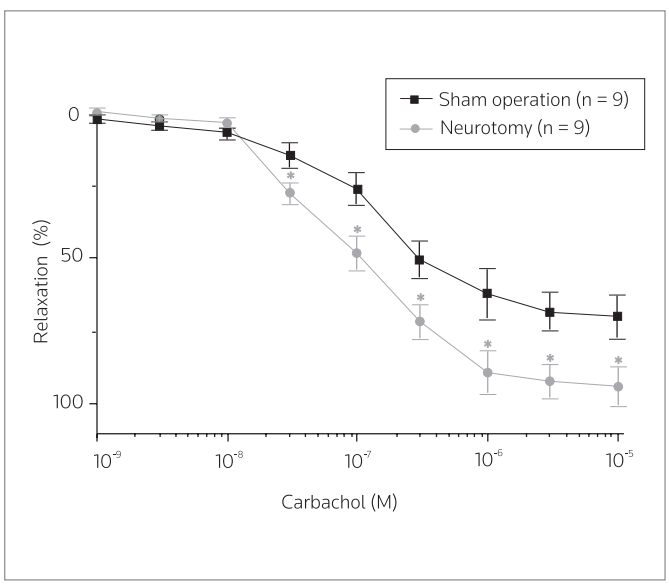


Figure 2. Carbachol concentration–response curves in isolated rabbit corpus cavernosum strips precontracted with phenylephrine 10 μM. Each point is expressed as a percentage of the contraction induced by phenylephrine and is given as the mean ± standard error of the mean (S.E.M.). Numbers in parentheses indicate the number of preparations used. *$P < 0.05$, statistically different from the response of strips from sham-operated rabbits.

Table I. *E_{max} (% of 10 μM phenylephrine) and pD_2 values (–log M) for carbachol, sodium nitroprusside and papaverine in corpus cavernosum strips obtained from neurotomy and sham operation groups. The table also denotes the E_{max} (% of 10 μM phenylephrine) value of electrical field stimulation (EFS) and the E_{max} value (mg) for 124 mM KCl.*

	Sham operation (n = 9)	Neurotomy (n = 9)
Carbachol		
E_{max}	68,4 ± 5.3	93.8 ± 6.8*
pD_2	6.5 ± 0.05	7.10 ± 0.06*
Sodium nitroprusside		
E_{max}	92.3 ± 2.6	88.4 ± 5.6
pD_2	6.20 ± 0.06	6.24 ± 0.07
Papaverine		
E_{max}	98.2 ± 1.8	96,6 ± 3,4
pD_2	4.58 ± 0.05	4,68 ± 0,06
EFS		
E_{max}	75.8 ± 5.1	38.4 ± 5.3*
KCl		
E_{max}	2094.83 ± 494.28	2115.95 ± 141.89

Values are arithmetic means ± S.E.M. n = the number of preparations used. *$P < 0.05$, statistically different from the response of strips from sham-operated rabbits.

cavernosal tissue from the neurotomized group was significantly reduced when compared to the sham-operated group ($P < 0.05$) (Fig. 3, Table I). The contractions elicited by 124 mM KCl were similar in both groups.

DISCUSSION

The present study demonstrates that a close relationship may exist between unilateral cavernous nerve neurotomy and the development of corporal smooth muscle dysfunction in rabbits. In this study, transmural electrical stimulation produced frequency-dependent relaxation in the isolated rabbit corpus cavernosum pretreated with guanethidine and atropine. According to our previous studies, stimulation-induced relaxation of rabbit corporal smooth muscle is abolished by pretreatment with 1 μM tetrodotoxin, and therefore this response is regarded to be a result of nerve stimulation (15, 16). We and other authors have also confirmed the nitrergic nature of the EFS-induced responses by studying the influence of NO synthase (NOS) inhibitors (8, 15-17).

Previously, iatrogenic impotence due to damage to the cavernous nerves has been recognized as a serious problem (5, 6). Later studies have emphasized the importance of an intact cavernous nerve in erectile control. Although authors have demonstrated that cavernous nerve ablation in rats resulted in no morphological or functional changes to the penile erectile tissue (18), it is further reported that rats with cavernous nerve transection were severely impaired in their attempt to effect intromission and failed to have erections with

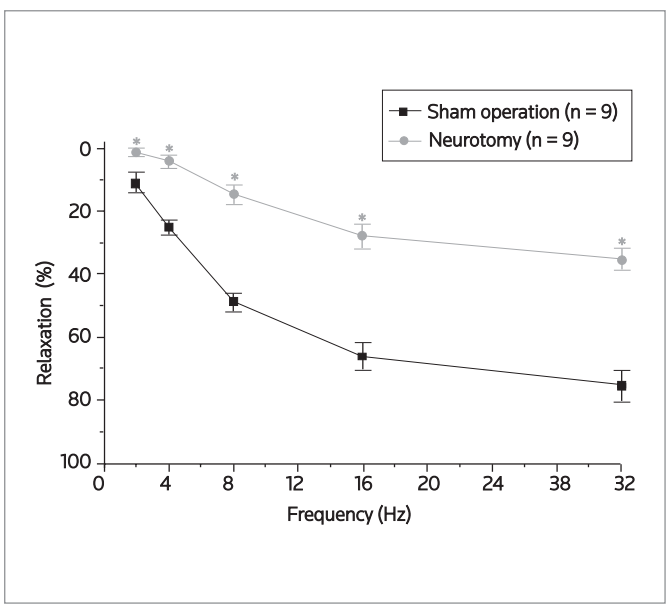


Figure 3. The relaxation responses evoked by electrical field stimulation of isolated rabbit corpus cavernosum strips precontracted with phenylephrine 10 μM. Each point is expressed as a percentage of the contraction induced by phenylephrine and is given as the mean ± standard error of the mean (S.E.M.). Numbers in parentheses indicate the number of preparations used. *$P < 0.05$, statistically different from the response of strips from sham-operated rabbits.

electrical stimulation of the pelvic nerve (19, 20). Short-term studies in dogs have also shown ultrastructural changes in cavernosal smooth muscle after cavernosal nerve ablation (21). In a report by Carrier et al., after unilateral cavernous nerve neurotomy, the number of NOS-containing nerve fibers within the corpus cavernosum decreased to a minimum on the neurotomized side after 3 weeks, but by 6 months the number had increased significantly and approximated the level on the controlateral side (22). Furthermore, Jung et al. noted that in the rat penis, 6 months after cavernous nerve neurotomy, the number of intracavernosal nerves with NOS-containing nerve fibers on the neurotomized side was restored to normal levels and pelvic ganglion stimulation in the unilateral neurotomized group revealed a greater maximal intracavernous pressure when compared to responses after 1 month (23). Also, Ayajiki et al. demonstrated that denervation for 1 week just distal to the pelvic plexus did not impair the relaxant response to EFS applied to isolated dog corpus cavernosum strips. In contrast, denervation close to the corpus cavernosum abolished the stimulation-induced response and also NADP diaphorase staining in the corpus cavernosum trabecula (24).

Our findings here are consistent with previous evidence. In the present study, although there were no histological differences, unilateral cavernous nerve neurotomy caused impaired EFS-induced neurogenic relaxation after 4 weeks, at all frequencies applied to the smooth muscle. This would suggest a possible common pathophysiological mechanism by which alterations in the NO/cGMP pathway, other pathways or neurotomy may impair the relaxation of trabecular smooth muscle or diminish its sensitivity to NO. However, such possibilities are unlikely since the corporal strips relaxed in response to sodium nitroprusside, which is metabolized by the smooth muscle to NO. The normal responses to sodium nitroprusside in the neurotomized rabbits therefore indicate a normal cGMP-dependent relaxation of corporal smooth muscle in these animals. In addition, there were no differences in the KCl-induced contractile responses between two groups. Thus, the contractile mechanisms were intact in the cavernosal smooth muscle. For this reason, it can be speculated that neurotomy impairs the synthesis or availability of NO in corpus cavernosum tissue.

An increasingly popular theory is that the formation of lipid peroxidation products has an important pathophysiological role in erectile dysfunction after unilateral cavernous nerve resection in rats. In recent years, authors have reported that unilateral cavernous nerve neurotomy caused oxidative stress and a decrease in intracavernosal pressure and NOS fibers in rat corpus cavernosum, and that they recovered 6 months after neurotomy (25). Therefore, it may be suggested that alteration of neurogenic relaxation may be the result of quenching and inactivation of NO by lipid peroxidation products.

Another finding of this study was that endothelium-dependent cavernosal relaxation in response to carbachol was enhanced in the neurotomized group. Previously, we and other authors have confirmed the nitrergic nature of the endothelium-dependent relaxation responses by studying the influence of NOS inhibitors (16, 26). Therefore, this feature of neurotomy involves adaptations specific to the endothelium, as suggested by two lines of evidence. Firstly, at the concentrations of phenylephrine used, the developed tension was similar in the trabecular strips from both groups, thus ensuring

that any difference in relaxation between neurotomized and sham control preparations was not due to differences in the degree of pre-contraction. Secondly, corporal relaxation responses to sodium nitroprusside were identical in the sham-operated and neurotomized rabbits. As sodium nitroprusside donates NO directly to smooth muscle, this finding indicates that the smooth muscle response to NO is not altered by neurotomy. Thus, the enhanced response to carbachol is not due to an enhanced response of corporal smooth muscle to NO.

In addition to these findings, the present study indicates that the relaxation responses to papaverine were similar in the two groups. This suggests that the increase in the endothelium-dependent relaxation response to carbachol in rabbits after neurotomy probably occurs at the level of the endothelium and not the smooth muscle cells, and is most likely to be due to the endothelial cell response to carbachol receptor-mediated activation. This finding is in agreement with previous studies in which NADPH diaphorase staining was more intense in corpus cavernosum muscles and endothelial cells in unilateral cavernous nerve-resected rats (27). Therefore, it is speculated that the enhanced response to carbachol in our study might be due to adaptive changes of eNOS in the endothelial cells within the sinusoidal spaces.

In conclusion, the present study has demonstrated that cavernosal neurotomy inhibits the noradrenergic noncholinergic (NANC)-mediated relaxation of the rabbit corpus cavernosum smooth muscle. The mechanism of neurotomy-induced dysfunction of corporal smooth muscle is unknown, but does not appear to involve alterations in the cGMP-dependent relaxation of corpus cavernosum smooth muscle. Alteration in the NANC-mediated relaxation of corporal smooth muscle in men with damaged cavernous nerve may, at least in part, contribute to the development of impotence. The development of this animal model will facilitate further investigation of the alterations of penile smooth muscle reactivity caused by cavernous nerve injury.

DISCLOSURES

The authors state no conflicts of interest.

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