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# Cavernosal Smooth Muscle Function After Experimental Ischemic Priapism

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**What's known on the subject? and What does the study add?**

Ischemic priapism (IP) is a kind of compartment syndrome which develops in penis. The role of apoptosis is defined for the compartment syndrome however, the role of apoptosis during and after priapism are not known. Also, the possible treatment with apoptosis inhibitors for the consequences of priapism is not known. This experimental study has yielded important results such as apoptosis have an important role for the etiology of erectile dysfunction after IP and new treatment insights for IP treatment.

## Abstract

**Objective:** We aimed to investigate the role of apoptosis and its effects on cavernosal smoothmuscle (CSM) function after ischemic priapism (IP) in a rat model.

**Materials and Methods:** Twelve adult Sprague-Dawley rats were assigned into two groups: The priapism and control groups. In all rats, erections were obtained by the vacuum method; however, in the priapism group, erections were maintained with a rubber band for 4 h to mimic priapism. After 14 days following this procedure, penile excision was performed and cavernosal tissues were investigated pharmacologically and histopathologically. Isometric tension changes due to several contractile and relaxant pharmacological agents were investigated with/without nitric oxide synthase (NOS) and guanylate cyclase (GC) inhibition.

**Results:** Isometric contraction and relaxation responses due to the agents applied did not differ between the groups. However, with NOS and GC inhibition, the cavernosal tissues relaxed less in the priapism group than in the controls ($p<0.05$). Histopathological evaluation of the tissues revealed that the average apoptosis rates were greater in the priapism group than in the controls in both the CSM (60.3% vs. 31.8%) and cavernosal epithelia (40.2% vs. 7%).

**Conclusion:** IP induction caused fibrosis by the apoptotic process in rat CSM. After IP, apoptosis in endothelial tissue was more evident than that in smooth muscle tissue in the corpus cavernosum. IP is likely to affect the NO pathway, resulting in a decrease in the NO-dependent relaxation in the cavernosal tissue.

**Keywords:** Apoptosis, erectile function, ischemic priapism, priapism

## Introduction

Priapism is defined as complete or partial penile tumescence that continues for 4 h with or without sexual stimulation (1). Priapism is categorized as ischemic priapism (IP) (veno-occlusive, low-flow), stuttering priapism (intermittent), and non-IP (arterial, high-flow) (1). Sexual stimulation triggers the release of neurotransmitters from the cavernous nerve terminals, resulting in penile erection in men (2). Penile erection involves sinusoidal relaxation, arterial dilation, and venous compression (2). Detumescence involves the opposite: Cavernosal smooth muscle (CSM) contraction, decreased arterial flow, and increased venous

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flow (3). Nitric oxide (NO) released from the noradrenergic/non-cholinergic nerves and endothelium is the principal neurotransmitter mediating penile erection (2). NO increases the production of cyclic guanylate monophosphate (cGMP), which in turn relaxes the CSM. Acetylcholine also contributes indirectly to penile erection (2).

Priapism may cause necrosis in the cavernosal tissue, which can lead to erectile dysfunction (4). IP can cause hypoxia, hypercarbia, and acidosis in the cavernosal tissue, which may lower adenosine triphosphate (ATP) levels and initiate the apoptotic process (5). This condition is analogous to muscle compartment syndrome, which has previously been well documented to cause histologic changes in the CSM within 12 h (1,6). The tone of the arterial and CSM cells is a key regulator of tumescence and detumescence and is determined by the balance between the relaxing and constricting factors (7). IP can cause necrosis in CSM, which may lead to resistance to $\alpha$-agonist treatment (5). However, the development of erectile dysfunction and CSM function after IP remains unclear.

This study aimed to evaluate the role of apoptosis and its consequences on CSM function after a single IP episode induction in a rat model.

## Materials and Methods

This study was approved by the Başkent University Institutional Review Board and Ethics Committee (project no: DA 06/39, date: 20.11.2006). The study was performed in 12 Sprague-Dawley adult (3 months and 10 days old) male rats (255.25±36.9 g), which were assigned into 2 groups (P: Priapism and C: Control). The number of rats calculated according to Festing and Altman's (8) guideline and assuming a significance level of 5%, a power of 90%, and a two-sided test revealed 5 animals per group. One more animal was added to the groups in case of animal loss due to surgical procedures. The rats were housed in standard rat cages under constant environmental conditions (20±2 °C room temperature, relative humidity 50±10%, light: dark cycle: 12-12 h) and were fed standard laboratory rat chow and tap water *ad libitum.* There was no blinding. Penile erection was induced by the vacuum method in both groups. In the control group, erection ended spontaneously within 5-10 min. To mimic priapism, erection was maintained for 4 h by constricting the base of the erected penis with a rubber band. Priapism was accomplished as previously described in the literature (9). En *bloc* penile excision was performed after 14 days of mimicked 4-h priapism. All surgical procedures were performed under anesthesia obtained by 10 mg/kg intraperitoneal (*ip*) xylazine (Rompun 2%, Bayer, Turkiye) and 60 mg/kg *ip* ketamine (Alfamine 10%, Ege Vet, Turkiye).

Penile cavernous tissues were placed in a dissection plate containing oxygenated, ice-cold Krebs-Henseleit solution and trimmed free of the surrounding connective tissue. The corpus cavernosum was divided and prepared for isolated organ bath (IOB) experiments. One of the parts was fixed in Bouine's solution for histopathological evaluation. The terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was used to investigate apoptotic parameters in the smooth muscle and cavernosal endothelium. The tunical continuity of the second cavernosal portion was broken by three incisions (upper-middle-lower) before the strips were transferred into the IOBs.

### IOB Experiments

The IOB system is a useful tool for examining isometric responses (contraction-relaxation) of a muscle-containing organ to various bioactive agents under physiological circumstances. Each corpus cavernosum strip was mounted longitudinally in a double-jacket glass IOB containing 10 mL of Krebs-Henseleit solution. The solution was maintained at 37 °C, pH 7, and aerated with carbogen (95% $O_2$ + 5% $CO_2$). To obtain the optimum isometric response, an initial resting tension of 1 g was applied to the tissues, which were allowed to equilibrate for 60 min without adding any test agent into the baths, during which the baths were washed out with fresh Krebs-Henseleit solution every 15 min. The isometric responses were perceived by a transducer (FT03, BioPac, MAY, Turkiye) connected to a computerized physiological data collection and acquisition system (Bio Pac, MP100, MAY, Turkiye).

The composition of the Krebs-Henseleit solution was as follows (mmol): NaCl, 118.2; KCl, 4.7; $MgSO_4$, 12; $CaCl_2$, 2.5; $KH_2PO_4$, 1.2; $NaHCO_3$, 25; and glucose, 11.1.

### Drugs and Test Agents Used

Phenylephrine (PE; $\alpha_1$-adrenoreceptor agonist; Sigma, St. Louis, USA), acetylcholine (Ach; Sigma), L-arginine [L-Arg; NO synthase (NOS) substrate; Sigma], sodium nitroprusside (SNP; exogenous NO donor; Sigma), Nitro-L-arginine methyl ester hydrochloride (L-NAME; inhibitor; Sigma), 1H-[1, 2, 4] Oxadiazolo [4, 3-a] quinoxalin-1-one [ODQ; guanylate cyclase (GC) inhibitor; Sigma].

All drugs were dissolved in distilled water. The mentioned concentrations are the final concentrations in the IOBs. The drugs were added to the IOBs cumulatively. The inhibitors and/or antagonists were added to the IOB 15-30 min before any agonist administration. Before adding the subsequent test agent, the baths were washed three times with fresh Krebs-Henseleit solution at 5-min intervals.

Concentration-contraction curves were obtained from the responses of the cavernosal strips to increasing logarithmic concentrations of the test agents. Each response was normalized

to the submaximal concentration (10 mmol/mL) of PE to avoid interindividual variations among preparations.

### Experimental Protocols

Logarithmic PE concentration-contraction curves were obtained for the cavernosal strips. Then, concentration-relaxation curves for ACh, L-Arg, and SNP were obtained by applying these agents at increasing consecutive concentrations to PE (0.1 mmol/L)-pre-contracted cavernosal strips. These protocols were repeated after incubating the IOBs for 15 min with L-NAME (1 mmol/L) or ODQ (1 mmol/L).

### Histopathological Evaluation

The cavernosal strips, fixated in Bouine's solution, were evaluated under a light microscope with routine hematoxylin and eosin (H&E) staining. The strips were also evaluated using the TUNEL method for apoptosis. The results of TUNEL staining expressed the percentage of smooth muscle cells and cavernosal endothelium cells with TUNEL-positive nuclei.

### Statistical Analysis

The randomization scheme for the rats according to the experimental groups was generated using a computer-based program (http://www.randomization.com). The data are expressed as the mean and standard error of the mean (SEM). Two-Way analysis of variance preceding post-hoc Bonferroni's test was used for the statistical analysis of the effects of priapism induction and/or the drugs applied. A p-value of 0.05 was considered statistically significant. The data were calculated with Microsoft Excel 2003 (Microsoft, WA, USA), and statistical analyses were performed using Graphad Prism v4.0 (GraphPad, CA, USA).

## Results

PE caused concentration-dependent contractions, whereas ACh, L-Arg, and SNP caused concentration-dependent relaxation in the cavernosal strips in both groups. The inhibition of neither nitric oxide synthase (NOS) nor guanylyl cyclase (GC) did not alter the relaxation induced by ACh, L-Arg, and SNP in the control group, whereas the concentration-dependent relaxation in response to ACh and L-Arg were decreased by NOS or GC inhibition in the priapism group (for the $E_{max}$ levels of ACh and L-Arg; -8.8±2.63% for NOS inhibition and -9.0±5.54 for GC inhibition vs. -18.9±1.47% for basal control; and -15.0±3.29% for NOS inhibition vs. -28.1±3.84% for basal control; respectively $p<0.05$; Figure 1). However, NOS or GC inhibition did not significantly affect SNP relaxation and PE contraction. The half maximal effective concentration ($EC_{50}$) values indicating the potency of PE are summarized in Table 1.

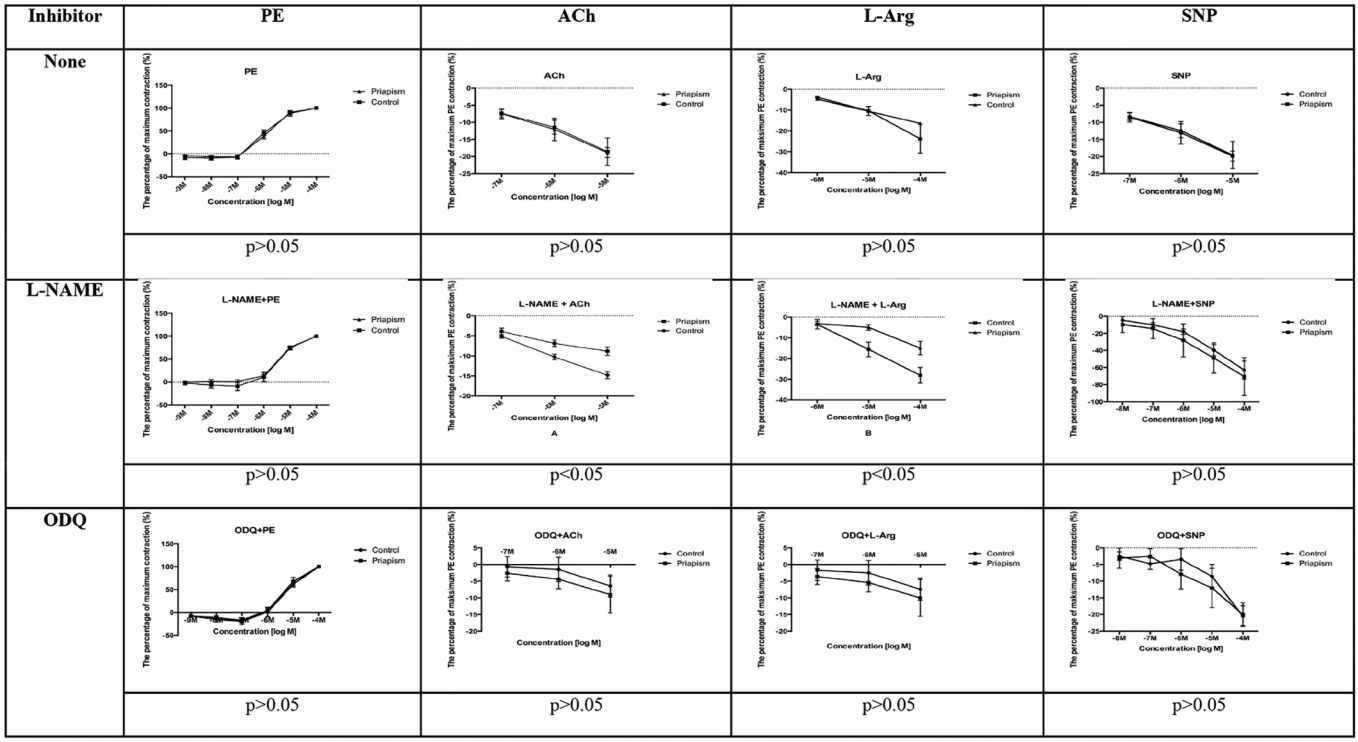


**Figure 1.** The contraction-relaxation responses due to test agents

PE: Phenylephrine, ACh: Acetylcholine, L-Arg: L-Arginine, SNP: Sodium nitroprusside, L-NAME: Nitro-L-arginine methyl ester (NOS inhibitor), ODQ: 1H-[1, 2, 4] Oxadiazolo [4, 3-a] quinoxalin-1-one (Guanylate Cyclase inhibitor)

| Table 1. Comparison of the half maximal effective concentration ($EC_{50}$) values of phenylephrine between the groups | | | | | | |
|---|---|---|---|---|---|---|
| | Basal | | L-NAME | | ODQ | |
| | $EC_{50}$ (mmol/mL) | log $EC_{50}$ | $EC_{50}$ (mmol/mL) | log $EC_{50}$ | $EC_{50}$ (mmol/mL) | log $EC_{50}$ |
| **Control** | 1.03 | -5.986 | 4.32 | -5.365 | 6.16 | -5.25 |
| **Priapism** | 1.4 | -5.855 | 4.64 | -5.334 | 5.21 | -5.283 |
| L-NAME: Nitro-L-arginine methyl ester hydrochloride (0.1 mmol/L), ODQ: 1H-[1, 2, 4] Oxadiazolo [4, 3-a] quinoxalin-1-one (0.1 mmol/L), $EC_{50}$: Half maximal effective concentration | | | | | | |

Histopathological examinations revealed that priapism induction increased the apoptotic parameters indicated by mean values of TUNEL-positive CSM and endothelial cells [60.3% vs. 31.8%, p=0.02 and 40.2% vs. 7%, p=0.02, respectively (see, Figure 2)].

## Discussion

IP is the most common form of priapism, accounting for >95% of all priapism episodes (1,10). According to our findings, the consequences of IP can be summarized in three headings.

### Cavernosal Fibrosis

IP causes CSM fibrosis, probably initiated by apoptosis. The apoptotic process is initiated with cavernosal ischemia and is followed by the reperfusion process as a typical compartment syndrome (4,11). If IP is not treated, it might cause penile necrosis (12). However, inappropriate or delayed treatment of IP can cause erectile dysfunction (1). The acting component of the cavernosal tissue during erection is the cavernosal and arterial smooth muscle (7). After priapism, the alteration of the erectile function depends on the degree of the affected CSM. Our data revealed that CSM fibrosis is encountered within 14 days of 4-h IP. The contraction and relaxation experiments showed that these functions of CSM were preserved in the priapism group compared with the controls. Thus, in our study, both functions of the CSM were preserved within a 4-h period of priapism. Muneer et al. (5) reported that CSM contractility was impaired within a 4-h period of hypoxia, acidosis, and glucopenia. They reported that there were no visible changes on CSM with H&E staining (5). Our data are in contrast with their report. This might be due to the *in vivo* nature of our experiment and/or the 14-day period of investigation after priapism induction, which might have provided enough time for the protective mechanism to maintain CSM function (13). Because of the *in vitro* study design of the study by Muneer et al. (5), it was not possible to show fibrosis. In addition, ischemia-reperfusion studies of cardiac muscle revealed that overall contractility of cardiac myocytes reduces in the early phases of reperfusion (14). After the reperfusion phase, myocytes develop hypertrophy in time to preserve cardiac contractility (15). Although cardiac muscle is not smooth muscle, this mechanism may explain how CSM contractility is preserved after 14 days of a 4-h period of priapism.

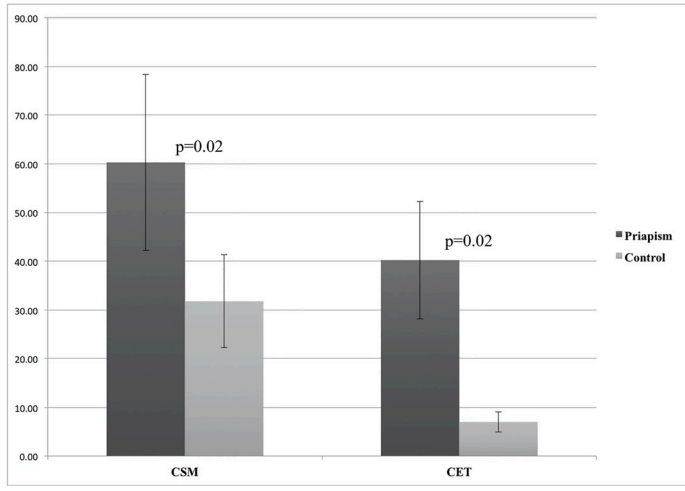


**Figure 2.** Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) positive nuclei rates of the priapism and control groups.

CSM: Cavernosal smooth muscle, CET: Cavernosal endothelial tissue, SD: Standard deviation

### Apoptosis

The apoptotic process after IP has been shown in several studies that have mainly focused on CSM apoptosis causing erectile dysfunction after IP (5,16). The TUNEL staining findings revealed that the priapism group had almost two-fold more TUNEL staining than the controls. The TUNEL- positive cells were 60.8% in the priapism group and 31.8% in the control group. It is obvious that IP caused apoptosis in the CSM, but our data showed that both functions of the CSM, contraction and relaxation, were preserved after IP for 4 h. This is in concordance with the daily clinical practice in which most IP cases, which last more than 4 h, can be treated with aspiration and irrigation.

Apoptosis in the control group and in the CSM (31.8%) was unexpectedly higher than that in previously reported studies. Muneer et al. (5) reported 1% TUNEL-positive CSM under *in vitro* control conditions. However, our results were obtained from *in vivo* experiments. The differences *in vivo* and *in vitro* apoptotic processes due to IP require further research to explain these results.

In the present study, apoptosis in the cavernosal endothelium was higher in the priapism group than in the control group. The TUNEL-positive cavernosal endothelium was 40.2% in the priapism group and 7% in the controls. The penile erection is initiated by

the release of NO from the cavernosal endothelium (2). Our data also elicited that the CSM functions were preserved; thus, the main cause of the ED after IP might be the destructed cavernosal endothelium. To the best of our knowledge, this is the first report on apoptosis of the cavernosal endothelium.

### NOS Malfunction

In the present study, one of the most obvious results was NOS malfunction of the cavernosal strips in the priapism group. The L-Arg-NOS-NO pathway is crucial in the physiology of erectile function, and its impairment is associated with the pathophysiology of priapism. NO produced by neuronal NOS (nNOS) within the nitrergic nerves is responsible for the initiation and most of the smooth muscle relaxation, whereby NO from endothelial NOS (eNOS) contributes to the maintenance of erection (2). However, exaggerated erectile responses have been reported in mice lacking the gene encoding for eNOS (17,18). Thus, this pathway might be responsible for the development of priapism as well. Several researchers have investigated the therapeutic effects of phosphodiesterase type-5 inhibitors (PDE-5i) on priapism (19,20). Bivalacqua et al. (19) have shown that in the absence of eNOS, PDE-5i can restore eNOS and prevent priapism. In the present study, in comparison with the control group, NOS inhibition significantly decreased the relaxation of the cavernosal strips in response to ACh in the priapism group. It has been reported that L-NAME is not selective for any NOS subtype, but as mentioned above, eNOS is the major NOS subtype responsible for maintaining the relaxation of the CSM tissue (2,21). In the present study, basal relaxation in response to ACh were similar in both groups; however, inhibition of NOS diminished the dysfunctional relaxation of the cavernosal strips only in the priapism group. This finding was also consistent with cavernosal endothelium apoptosis. Nevertheless, further research is needed to evaluate the role of eNOS in both erectile function and IP, especially focusing on non-adrenergic non-cholinergic signaling and NO-dependent relaxation in the cavernosal tissue.

Contrary to our expectations, in both groups, L-NAME or ODQ incubations shifted the PE concentration-contraction curves rightward, indicating the inhibitory role of NOS or GC inhibition on the contractility of the corpus cavernosum. It might be speculated that this surprising finding is due to the effect of peroxynitrite, a non-enzymatic product of the NO and superoxide reactions. It has been reported that peroxynitrite can contract the vascular smooth muscle (22). However, because the $EC_{50}$ value of PE-induced contractions was not significantly changed by NOS or GC inhibition, it is early to propose a mechanistic explanation, and it remains to be established by further research.

### Study Limitations

Our study has three limitations. First, we did not evaluate the rate of fibrosis. Although histological changes after priapism have been reported, the exact rates of fibrosis both in the CSM and endothelium might be more explanatory. Second, the expression levels of NO-cGMP pathway enzymes were not evaluated. Third, we evaluated the CSM functions. Although CSM relaxation and sinusoidal relaxation are important steps of tumescence/erection, the whole process of erectile function cannot be interpreted just with CSM functions.

## Conclusion

IP induction caused fibrosis in the CSM of rats, which was probably initiated by the apoptotic process due to ischemia. After IP, apoptosis in endothelial tissue was more evident than that in smooth muscle tissue in the corpus cavernosum. IP is likely to affect the NO pathway, resulting in a decrease in the NO-dependent relaxation of the cavernosal tissue.

### Ethics

**Ethics Committee Approval:** This study was approved by the Başkent University Institutional Review Board and Ethics Committee (project no: DA 06/39, date: 20.11.2006).

**Informed Consent:** Not necessary.

### Authorship Contributions

Surgical and Medical Practices: C.Ö., M.R.G., Concept: E.K., S.R.E., M.R.G., Design: S.R.E., M.R.G., Data Collection or Processing: E.H., C.Ö., M.R.G., Analysis or Interpretation: E.H., C.Ö., S.R.E., Literature Search: E.H., E.K., Writing: E.H., E.K.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declare that they have no relevant financial.

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