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Effects of chronic unilateral internal pudendal arterial occlusion on reactivity of isolated corpus cavernosum strips from rabbits

Tijen Utkan [a,*], Yusuf Sarıoğlu [b], Nihat Zafer Utkan [c], Füsun Kurnaz [b], Sahin Yildirim [b]

[a] *Department of Pharmacology, Faculty of Medicine, University of Kocaeli, 41900, Kocaeli, Turkey*
[b] *Department of Pharmacology, Faculty of Medicine, University of Cumhuriyet, 58140, Sivas, Turkey*
[c] *Department of General Surgery, Faculty of Medicine, University of Kocaeli, 41900, Kocaeli, Turkey*

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Abstract

An animal model was developed to elucidate the effect of chronic obstruction of the internal pudendal artery on the responsiveness of the corpus cavernosum. In male albino rabbits, the internal pudendal artery was chronically ligated unilaterally with a silk tie and the occlusion was maintained for 1 month. The control group was sham-operated. The reactivity of corpus cavernosum tissue from the ligated animals and the control animals was studied in organ chambers. Unilateral chronic ligation of the internal pudendal artery caused an impaired contractile response to α-adrenoceptor stimulation with decreased E_m and pD_2 values and an impaired relaxant response to electrical field stimulation but resulted in a marked increase in the endothelium-dependent relaxant response to carbachol with an increased pD_2 value. However chronic obstruction of the pudendal artery had no effect on adenosine-, papaverine- and sodium nitroprusside-induced relaxant responses, and there was no change in agonist potency. These data indicate that altered penile hemodynamics have an effect on the reactivity of the corpus cavernosum and may contribute to the etiology of impotence.

Keywords: Ischemia; Corpus cavernosum; Contraction; Relaxation

1. Introduction

The precise mechanisms of penile erection are not yet fully understood, but it has been demonstrated that arterial flow, cavernosal smooth muscle relaxation and cavernosal outflow restriction are important components in penile erection (Fournier et al., 1987; Aboseif et al., 1990). The relaxation of the cavernous smooth muscle is largely nerve-mediated by a nonadrenergic, noncholinergic (NANC) mechanism (Saenz de Tejada et al., 1988); however, endothelium-dependent cholinergic neurotransmission may also mediate penile erection (Trigo-Rocha et al., 1993). Recent studies have shown that nitric oxide (NO) is responsible for the relaxation of rabbit and human corpus cavernosum smooth muscle strips (Ignarro et al., 1990; Knispel et al., 1991; Bush et al., 1992; Rajfer et al., 1992). Conversely, penile flaccidity is thought to be related to the release of norephinephrine from sympathetic nerve terminals and contraction of corporal smooth muscle subsequent to the activation of postsynaptic α_1-adrenoceptors (Benson et al., 1980; Andersson and Holmquist, 1990; Christ et al., 1990). Normal erectile function is characterized by a delicate balance in vivo between the effects of vasoconstricting and vasorelaxing hormones on corporal smooth muscle tone (Taub et al., 1993).

Many studies have shown that inadequate penile arterial flow is one of the major causes of impotence (Kedia, 1983; Virag, 1985). The first association between arterial insufficiency and erectile impotence was made by Leriche (1940). Recent in vivo studies have confirmed the importance of arterial occlusion in penile erection (Aboseif et al., 1990; Rosen et al., 1990; Vardi and Siroky, 1993; Azadzoi et al., 1996). Common risk factors associated with arterial insufficiency and erectile dysfunction include hypertension, hyperlipidemia, cigarette smoking and diabetes mellitus (Krane et al., 1989). The internal pudendal arteries, branches of the hypogastric arteries, carry blood to the penis, from where it is distributed to the corpus cavernosum, corpus spongiosum, and skin by deep and superfi-

* Corresponding author. Fax: +90-262-2335463; E-mail: tijenutkan@superonline.com

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cial penile arteries. The purpose of this study was to determine the effects of arterial occlusion-induced cavernosal ischemia on the reactivity of penile corporal smooth muscle. Strips of corpus cavernosum tissue from control and chronically occluded animals were used to compare the responses to constrictor and to endothelium-dependent and endothelium-independent dilator agents.

2. Material and methods

Mature male albino rabbits weighing 2.5 to 3 kg were used. The albino rabbit was chosen as the animal model based on the close similarities that have been reported in the reactivity in vitro of human and rabbit corpus cavernosum (Azadzoi and Goldstein, 1992). The animals were divided into two groups: group 1 served as control ($n = 10$) and underwent a sham-operation; group 2 was ligated ($n = 12$). Unilateral internal pudendal arterial occlusion was performed under aseptic conditions and under anesthesia induced by subcutaneous ketamine (50 mg/kg) and xylazine (5 mg/kg). In group 1 and 2, an incision was made from the xiphoid process to the pubic bone, the abdomen was opened, the left pudendal artery was ligated and the wound was closed. In the control group the artery was mobilized but left intact.

2.1. Corpus cavernosum tissue

One month later the rabbits were killed with a subcutaneous injection of ketamine and xylazine and exsanguinated. The penis was removed. A ventral incision was made on the right and left corpora, the tunica was dissected and the corpus cavernosum tissue was exposed. After dissection the corporeal tissue was immediately placed in organ chambers.

2.2. Organ chamber experiments

Strips of corpus cavernosum tissue measuring approximately 2 mm × 2 mm × 15 mm were mounted in 20-ml organ chambers for isometric tension measurements. Each rabbit provided 2–4 strips of corpus cavernosum smooth muscle which were studied separately. The strips were tied with silk to a force transducer (Grass FT 03, Quincy MA) on one end and fixed with silk ties to a glass support on the other end. The transducer output was recorded on a Grass polygraph model 79 E. The organ chambers contained Krebs-bicarbonate solution composed of (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgSO_4$ 1.2, $KHPO_4$ 1.2, glucose 11. 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 which was previously found to be optimal for measurement of changes in the tension of rabbit corpus cavernosal preparations (Yildirim et al., 1997). The preparations were allowed to equilibrate in Krebs-bicarbonate for 1 h and during this time Krebs-bicarbonate was replaced every 15 min with fresh solution. At the end of the equilibration period, strips were depolarized with 124 mM KCl in Krebs-bicarbonate solution and allowed to equilibrate for 30 min. This procedure increases and stabilizes subsequent contractile responses to phenylephrine and decreases spontaneous contractile activity. Af-

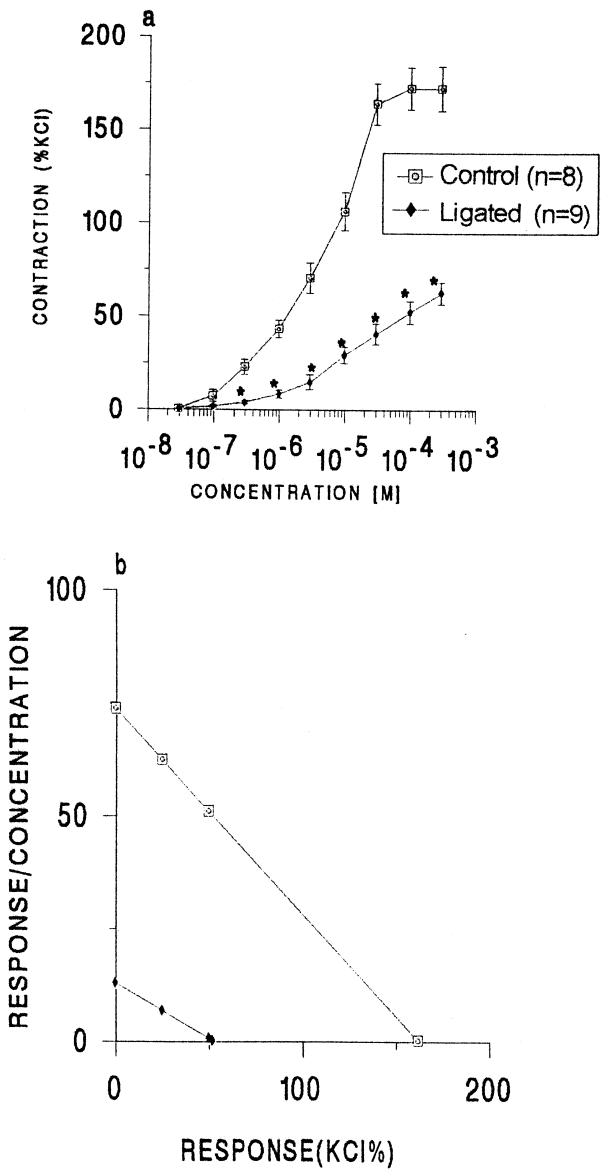

Fig. 1. (a) Phenylephrine concentration–response curves in isolated rabbit corpus cavernosum strips. Each point is expressed as a percentage of the contraction induced by 124 mM KCl and is given as the mean ± S.E.M. Numbers in parentheses indicate the number of preparations used. * $P <$ 0.05, statistically different from the response of strips from control rabbits. (b) Scatchard analysis. E_{max}% and pD_2 values shown in Table 1Table 2 ($r = 0.86$ for control and $r = 0.69$ for arterial occlusion).

Table 1
E_{max} (percentage of 10^{-5} M phenylephrine) values for carbachol, adenosine, sodium nitroprusside, papaverine, and EFS and E_{max} values (percentage of 124 mM KCl) for phenylephrine in corpus cavernosum strips obtained from artery ligated and control rabbits

	Control	n	Artery-ligated	n
Carbachol	67.90 ± 8.72	8	94.90 ± 7.66[a]	9
Sodium nitroprusside	92.69 ± 6.74	8	94.70 ± 4.56	9
Adenosine	73.60 ± 5.24	8	66.50 ± 8.96	9
Papaverine	99.2 ± 1.30	8	97.8 ± 2.10	9
EFS	72.40 ± 4.56	8	56.30 ± 4.38[a]	9
Phenylephrine	160 ± 9.7	8	51.2 ± 7.8[a]	9

Values are arithmetic means ± S.E.M., n = number of observations.
[a] $P < 0.05$, statistically different from the response of strips from control rabbits.

ter equilibration, the contractile responses to phenylephrine (10^{-8} to 10^{-4} M) were obtained cumulatively. After the addition of each dose, we waited until a plateau response was obtained before adding the next one. After a 30-min washout period, the strips were contracted again with a submaximal concentration of phenylephrine (10^{-5} M) and concentration–response relationships for carbachol (10^{-9}–10^{-4} M), adenosine (10^{-6}–10^{-4} M), papaverine (10^{-5}–10^{-4} M) or sodium nitroprusside (10^{-8}–10^{-5} M) were obtained by adding one of these agents to the bath in a cumulative manner.

Electrical stimulation at supramaximal voltage (50 V) was performed through two platinum electrodes mounted parallel to the corporal strip, using a Grass stimulator with constant current output. Square pulses of 0.8 ms duration in 10-s trains with varying frequency (2, 4, 8, 16 and 32 Hz) were applied at 5-min intervals. A first frequency–response curve for phenylephrine-contracted preparations was obtained. Then the strips were allowed to return to baseline precontractile tension between the tests at each frequency. Subsequently, without the strips being washed, *N*-nitro-L-arginine methyl ester (L-NAME) (3×10^{-5} M) was added to the strips and stimulation was repeated 15 min later. Guanethidine (5 μM) and atropine (1 μM) were present throughout the experiments with electrical field stimulation to inhibit adrenergic and cholinergic neurotransmission, respectively. Three or four agonists were tested on each preparation.

Table 2
pD_2 values for phenylephrine, carbachol, adenosine, papaverine, and sodium nitroprusside in corpus cavernosum strips obtained from artery ligated and control rabbits

	Control	n	Artery ligated	n
Phenylephrine	5.66 ± 0.11	8	5.40 ± 0.09[a]	9
Carbachol	6.95 ± 0.12	8	7.13 ± 0.08[a]	9
Sodium nitroprusside	5.73 ± 0.28	8	5.80 ± 0.22	9
Papaverine	4.26 ± 0.05	8	4.17 ± 0.04	9
Adenosine	3.95 ± 0.08	8	4.06 ± 0.09	9

Values are arithmetic means ± S.E.M., n = number of observations.
[a] $P < 0.05$, statistically different from the response of strips from control rabbits.

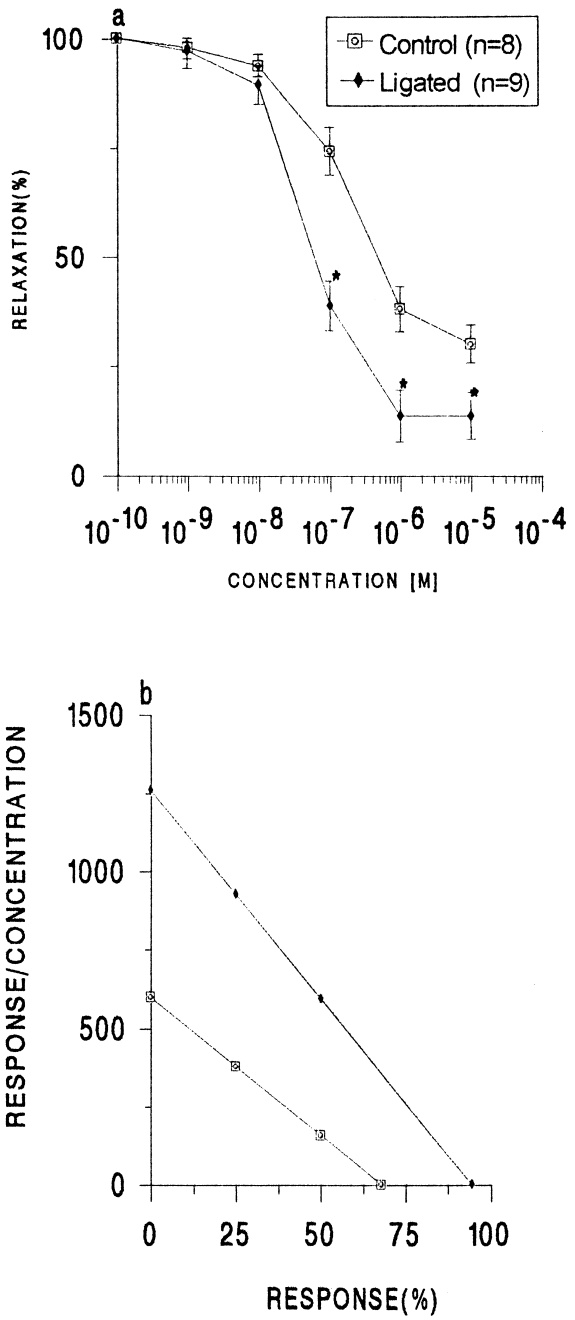

Fig. 2. (a) Carbachol concentration–response curves in isolated rabbit corpus cavernosum strips precontracted with phenylephrine 10^{-5} M. Each point is expressed as a percentage of the contraction induced by phenylephrine and is given as the mean ± S.E.M. Numbers in parentheses indicate the number of preparations used. * $P < 0.05$, statistically different from the response of strips from control rabbits. (b) Scatchard analysis. E_{max}% and pD_2 values shown in Table 1Table 2. ($r = 0.91$ for control and $r = 0.94$ for arterial occlusion).

2.3. Analysis of data and statistics

Experimental values are expressed as the means ± S.E.M. Contractile responses to phenylephrine were calculated as percentages of the maximal contraction caused by potassium (124 mM). Relaxant effects of agonists are expressed as percentages of the precontraction caused by phenylephrine. In order to evaluate the effects of agonists, maximum responses (E_m) and pD_2 values (apparent agonist affinity constants; $-\log ED_{50}$) were calculated. The concentration–response data obtained in each individual experiment were plotted as the response/concentration (y) against the response (x). This produced a straight line relationship for each experiment, as predicted from the Scatchard equation for drug–receptor interactions.

Statistical comparisons between groups were performed by using the unpaired Student's *t*-test. Probabilities of less than 5% ($P < 0.05$) were considered significant.

2.4. Drugs

The following drugs were used: Adenosine (Sigma), acetylcholine chloride (Sigma), phenylephrine hydrochloride (Sigma), *N*-nitro L-arginine methyl ester (L-NAME) (Sigma), sodium nitroprusside (Adeca), atropine sulfate (Sigma), guanethidine sulfate (Sigma), carbachol chloride (Sigma), papaverine hydrochloride (Sigma). All drugs were dissolved distilled water. L-NAME was initially dissolved in distilled water and frozen. On the day of use it was thawed and diluted in distilled water. All other drugs were freshly prepared on the day of the experiments.

3. Results

The cumulative addition of phenylephrine produced concentration-dependent contractions of the cavernosal strips and the contractility was significantly lower in the strips from ligated rabbits ($P < 0.05$). The concentration–response curve for phenylephrine was shifted to the right, with significantly lower E_m and pD_2 values in the ligated group than in the control group ($P < 0.05$) (Fig. 1, Tables 1 and 2). The contractions elicited by 124 mM KCl were similar in the two groups (data not shown).

Carbachol, adenosine, papaverine and sodium nitroprusside produced concentration-dependent relaxation in submaximally (60–70% of maximal contraction) precontracted (10^{-5} M phenylephrine) corpus cavernosum strips obtained from control and ligated rabbits. The relaxation in response to carbachol was significantly greater in strips from ligated rabbits than in strips from the control rabbits ($P < 0.05$). The concentration–response curve for carbachol was shifted to the left with significantly higher pD_2 values ($P < 0.05$) (Fig. 2, Tables 1 and 2). The relaxation elicited by adenosine, papaverine and sodium nitroprusside was similar in ligated and control groups and there were no significant changes in the pD_2 values (Tables 1 and 2). The nitric oxide synthesis inhibitor, L-NAME (3×10^{-5} M), significantly attenuated carbachol-induced relaxations in rabbit tissue. In contrast, the adenosine-induced relaxations were not reduced in the presence of L-NAME (data not shown). In precontracted strips, electrical field stimulation (2–32 Hz) evoked frequency-dependent relaxation. Treatment with L-NAME (3×10^{-5} M) increased basal tone in all strips and abolished the relaxations elicited at the lowest frequencies, while residual relaxations were still

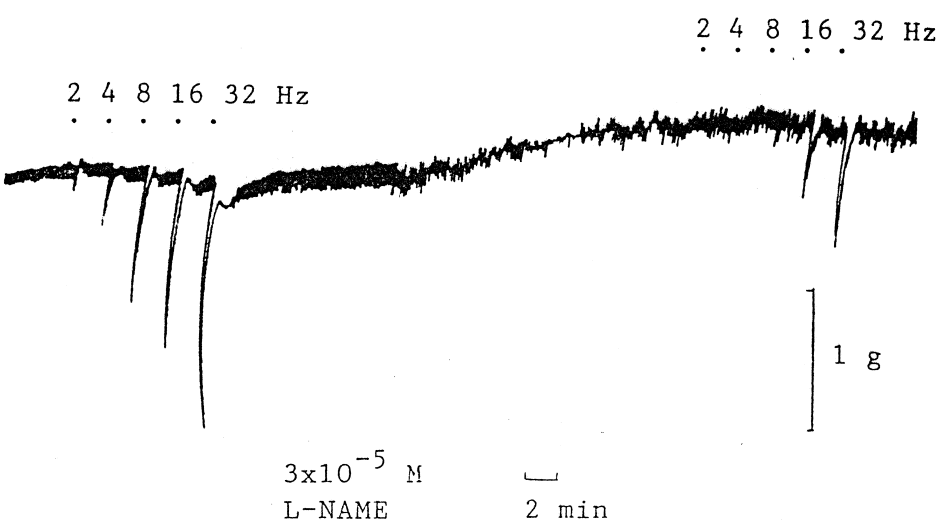

Fig. 3. The effect of electrical field stimulation (EFS) in isolated rabbit corpus cavernosum strips precontracted with phenylephrine 10^{-5} M and the inhibitor effect of L-NAME (3×10^{-5}) M in the presence of guanethidine (5 μM) and atropine (1 μM).

observed at high frequencies (16–32 Hz) (Fig. 3). In the ligated groups, all electrical field stimulation responses were lower than those in the control group ($P < 0.05$) (Fig. 4, Table 1).

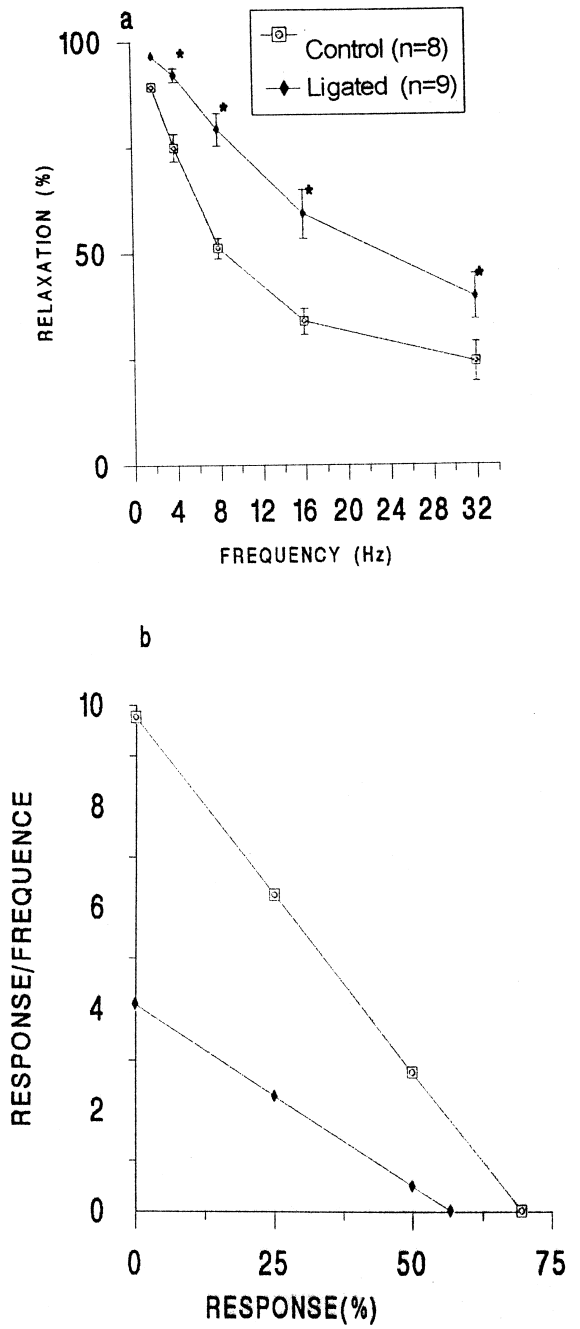

Fig. 4. The relaxation responses evoked by EFS of isolated rabbit corpus cavernosum strips precontracted with phenylephrine 10^{-5} M. Each point is expressed as a percentage of the contraction induced by phenylephrine and is given as the mean ± S.E.M. Numbers in parentheses indicate the number of preparations used. $* P < 0.05$, statistically different from the response of strips from control rabbits. (b) Scatchard analysis. E_{max}% values shown in Table 1. ($r = 0.88$ for control and $r = 0.78$ for arterial occlusion).

4. Discussion

Our results suggest that a close relationship may exist between unilateral arterial ligation-induced occlusion of the pudendal artery and development of corporal smooth muscle dysfunction in the rabbit.

Studies of rabbit, rat and human cavernosal smooth muscle (Ignarro et al., 1990; Knispel et al., 1991; Bush et al., 1992; Rajfer et al., 1992) in vitro suggest a role for nitric oxide as the neurotransmitter mediating relaxation. Recent studies suggest that NO mediates both neurogenic and endothelium-dependent relaxation of trabecular smooth muscle (Ignarro et al., 1990; Bush et al., 1992; Rajfer et al., 1992). Immunocytochemical localization of NO-synthase has indicated the presence of nitrergic nerves in corporal tissue (Burnett et al., 1992). In this study, transmural electrical stimulation produced frequency-dependent relaxation in the isolated rabbit corpus cavernosum pretreated with guanethidine and atropine. In addition, it has been reported that stimulation-induced relaxation of rabbit corporal smooth muscle is abolished by treatment with 3×10^{-7} M tetrodotoxin, and thus the response is regarded to result from nerve stimulation (Rajfer et al., 1992; Azadzoi and Goldstein, 1992). In the present study, the inhibition of NO synthesis from L arginine by means of L-NAME markedly reduced the electrically induced relaxations in corporal tissue. This result indicates that corporal tissue receives a functional nitrergic innervation, and that NO or its analog plays an important role in transmitting information from the nerve to the cavernous smooth muscle. However, in the present study treatment with a high concentration of L-NAME did not completely abolish the responses to electrical field stimulation at high frequencies in the rabbit corpus cavernosum tissues. This indicates that a nonadrenergic noncholinergic inhibitory neurotransmitter different from NO and probably of pepdidergic nature is released, because the relaxations were resistant to L-NAME preferentially at high frequencies of stimulation. In fact, several agents have been suggested to mediate the relaxation associated with penile erection such as vasoactive intestinal polypeptide (Andersson et al., 1984), and thus further studies in animal models must clarify the nature of the transmitter released by high-frequency stimulation.

Relaxation of corporal smooth muscle is required for penile erection as it allows the expansion of the lacunar spaces and reduces cavernosal venous outflow by compressing the venules against the tunica albuginea, the surrounding fibrous structure (Krane et al., 1989). In recent years arterial insufficiency of the corpus cavernosum has been recognized as an important cause of human impotence (Virag, 1985; Azadzoi et al., 1996, 1997). Arterial occlusion has been documented to cause diminished arterial perfusion pressure and decreased arterial inflow to the intracavernosal spaces (Bookstein et al., 1987; Krane et al., 1989; Aboseif et al., 1990; Rosen et al., 1990; Azadzoi and Goldstein, 1992). Decreased arterial blood flow may result

in an inability to expand the trabeculae against the tunica albuginea and compress the subtunical venules. In previous studies, it has been shown that hypercholesterolemia and atherosclerotic occlusive disease of the iliac arteries cause erectile dysfunction in the rabbit (Azadzoi and Goldstein, 1992). In the canine model, acute bilateral occlusion of penile arteries caused a 60% decrease in intracavernosal pressure during neurostimulated erection (Aboseif et al., 1989). In contrast, chronic mechanical ligation of the penile vessels had only a minimal effect on the erectile function due to the development of a rich network of collaterals around the penis (Houttuin et al., 1978; Aboseif et al., 1989). The observation in this study that arterial occlusion impaired neurogenic relaxation of trabecular smooth muscle would suggest a possible common pathophysiologic mechanism by which alteration of the nitric oxide/cGMP pathway or occlusion may impair the relaxation of trabecular smooth muscle or diminish its sensitivity to NO. However, these possibilities are unlikely since the corporeal strips relaxed in response to papaverine and to the NO-producing vasodilator, sodium nitroprusside. Sodium nitroprusside is a vasodilator and acts via the release of NO as an active metabolite within the smooth muscle cell. NO causes the activation of guanylate cyclase and the intracellular accumulation of cGMP (Krane et al., 1989; Rajfer et al., 1992). This inhibits the contractile process and results in the relaxation of corpus cavernosum smooth muscle. Therefore it is possible that occlusion leading to ischemia impairs the synthesis or availability of nitric oxide in corpus cavernosum tissue. This is consistent with the results of previous reports on dogs with cavernosal ischemia (Bookstein et al., 1987).

Based on studies of several species, including humans, NO, released by the penile endothelium, appears to play a major role in the initiation and maintenance of tumescence (Ignarro et al., 1990; Trigo-Rocha et al., 1993). Some studies suggest that the cholinergic stimulation of muscarinic receptors on endothelial cells releases NO, thus creating a common pathway for cavernosal relaxation (Knispel et al., 1991). The relaxation of human corporal smooth muscle in response to acetylcholine requires the presence of an intact endothelium (Saenz de Tejada et al., 1988). Adenosine has also been shown to affect smooth muscle relaxation via a cAMP-dependent mechanism (Katz, 1988). Endothelium-dependent cavernosal relaxation in response to carbachol increased in this study. However, the magnitude of the endothelium-independent relaxant response to adenosine in the occlusion group was similar to that of the control group. Because the response to the NO donor sodium nitroprusside was the same in cavernosal tissue from control and ligated rabbits, it may be assumed that the responsiveness of the erectile tissue to the effects that are mediated by NO was unchanged. It is possible that the increased response to carbachol in strips from ligated rabbits might be due to adaptive alterations in muscarinic receptors on the endothelial cells of the sinusoidal spaces.

Adrenergic nerve fibers and α and β-adrenoceptors have been detected in the cavernous trabeculae and surrounding the cavernous arteries, and noradrenaline is generally accepted as the principal neurotransmitter to control penile flaccidity and detumescence (Hedlung and Andersson, 1985; Diederichs et al., 1990). In corporeal smooth muscle, the receptors predominantly are of the α_1 type but α_2-adrenoceptors also exist (Benson et al., 1980; Christ et al., 1990). These α_1-adrenoceptors mediate the response to noradrenaline released from postganglionic nerve endings, resulting in smooth muscle contraction. Recent pharmacological studies with corporal tissue strips isolated from men with organic erectile dysfunction have demonstrated significant age-dependent alterations in the sensitivity and maximum amplitude of contractions elicited by phenylephrine (Christ et al., 1990, 1991). In a previous study, it was reported that phenylephrine-induced contractions decreased in cavernosal tissue from vasculogenic impotent men (Pickard et al., 1994). Likewise, in this study, the contractile response to phenylephrine was decreased in both sensitivity and maximal response in strips from the occlusion group. Evidence for a reduction in α-adrenoceptor-mediated contraction in ischemia was provided by Vanarsdalen et al. (1983), who showed such a reduction in the urinary bladder of rabbits. The decreased contractile response to α_1-adrenoceptor stimulation by phenylephrine found in the occlusion group in the present study requires interpretation in the light of our findings of a marked impairment of electrically evoked relaxation but normal relaxation in response to sodium nitroprusside and papaverine in the same group. Since there was no difference in the response to KCl between the occlusion group and the control group, the contractile mechanisms were intact in the cavernosal smooth muscles. Hence, the decreased response to phenylephrine in the occlusion group may be an adaptive phenomenon at the level of neurotransmitter receptors. The decreased α_1-adrenoceptor density after ischemia may be due to the down-regulation of α-adrenoceptors. It is possible that, clinically, the inability to contract corporal smooth muscle impedes drainage of ischemic blood accumulated in the corpora, which leads to further ischemia.

In conclusion, our findings suggest that arterial occlusion-induced cavernosal ischemia may impair the responsiveness of corporal smooth muscle, leading to impotence, and that ischemia affects smooth muscle function at a presynaptic or postsynaptic level.

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