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Nitric oxide mediates relaxation in rabbit and human corpus cavernosum smooth muscle

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Summary. We investigated in vitro the relaxant effect of exogenous acetylcholine (ACh) and electric-field stimulation (EFS) on rabbit and human corpus cavernosum smooth muscle strips (CC) precontracted with phenylephrine. The effects of EFS and ACh were monitored alone, after muscarinic receptor blockade and after inhibition of nitric oxide (NO) formation with L-*N*-nitroarginine (L-NOARG). In rabbit und human CC, both atropine and L-NOARG abolished the relaxant effects of ACh. The relaxant effects of EFS, however, were only slightly reduced by atropine to 97.5 ± 17.5% in human CC and to 89.0 ± 6.1% in rabbit CC. L-NOARG further reduced the EFS effects to 0.8 ± 1.7% in human CC and to 16.2 ± 8.7% in rabbit CC. In strips obtained from impotent patients with diabetes mellitus, the relaxant effects appeared to be significantly less than in strips from nondiabetic impotent men. Tetrodotoxin blocked the relaxant EFS effects in human and rabbit strips completely. The data indicate the important role of NO in cholinergically induced relaxation of cavernous smooth muscle in rabbits and humans. Our findings support the idea of NO as the nonadrenergic noncholinergic neurotransmitter in penile erection in both species. Rabbit erectile tissue might serve as an in vitro animal model for further investigation.

Key words: Corpus cavernosum – Nitric oxide – Neurotransmission – Penile erection – Smooth muscle relaxation

Impairment of corpus cavernosum smooth muscle (CC) relaxation is a common cause of organic impotence [16]. Therefore, numerous studies have focused on possible mediators of smooth muscle relaxation. The search for a nonadrenergic noncholinergic (NANC) neurotransmitter was prompted by the persistence of neurogenically mediated responses after adrenergic and cholinergic blockade [21]. Initially, prostanoids [10], vasoactive intestinal polypeptide (VIP) [1], calcitonin-gene-related peptide (CGRP) [25], and acetylcholine (ACh) [24] were suggested as important mediators in penile erection. However, the effects of those mediators, particularly those of acetylcholine (ACh) [24], were inconsistent and seemed to depend on species; an adequate animal model was lacking.

Recent studies on vascular smooth muscle have shown that nitric oxide (NO) plays a major role as a physiologic relaxant agent [19]. Our study was done to investigate the possible role of NO in cholinergically and neurogenically induced relaxation of CC by inhibiting NO formation. Comparison of results with human and rabbit erectile tissue should be useful for evaluating the rabbit as an animal model.

Materials and methods

Thirty-two male New Zealand white rabbits weighing 2–3 kg each were killed by a blow to the neck. Both corpora cavernosa of each rabbit were dissected in chilled Krebs' solution, and smooth muscle strips measuring approximately 2 × 3 × 10 mm were obtained. Similarly, cavernous smooth muscle strips were taken from 10 men who underwent implantation of penile prostheses. Each strip of rabbit and human tissue was mounted between two metal hooks in an organ-bath chamber containing 40 ml of a Krebs' solution with the following composition: NaCl, 118.1 mmol/l; $NaHCO_3$, 25.0 mmol/l; KCl, 4.6 mmol/l; KH_2PO_4, 1.2 mmol/l; $CaCl_2$, 2.5 mmol/l; $MgSO_4$, 1.2 ,mmol/l; glucose, 11.0 mmol/l. The solution was aerated with 5% CO_2 at 37°C; the resulting pH was 7.4.

Tension measurement and electric-field stimulation

The tension of each strip was measured isometrically while one hook was connected to a Hugo Sachs F 30 Type 372 force transducer. We used a Linseis LS-52-4 polygraph for recording. Electric-field stimulation was delivered by a TB-134 Pulsar 4 digital stimulator (Frederick Haer) connected to two platinum electrodes (1 mm in diameter, 2 mm long, and 5 mm apart) that were parallel to the tissue strip.

Drugs

We used the following drugs: L-phenylephrine hydrochloride (PE) and acetylcholine (ACh) chloride from Serva; atropine methyl-

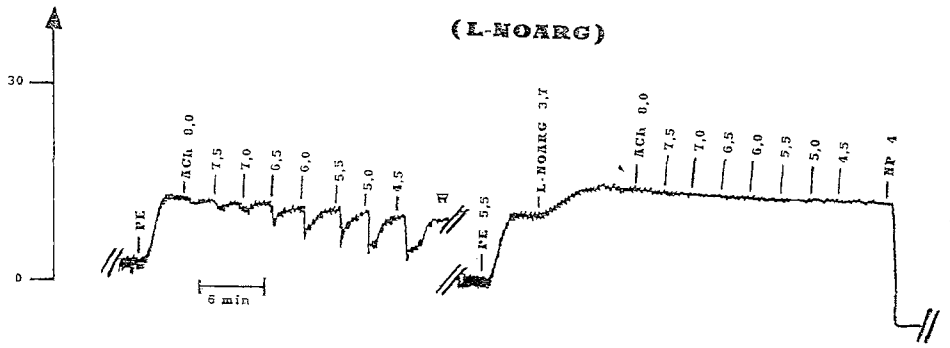

Fig. 1. Effects of ACh applied cumulatively on the tension of PE-precontractet *rabbit* cavernous smooth muscle strips alone (*left*) and after inhibition (*right*) by incubation with L-NOARG. *Numbers* after the substances are negative logarithms of their bath concentration. *W.* Washout, followed by a 45 min equilibration

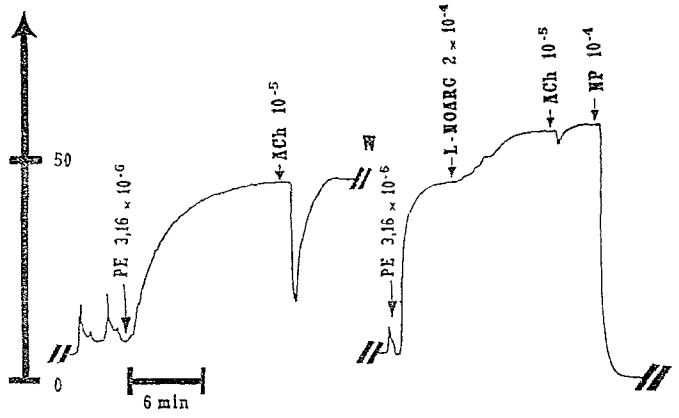

Fig. 2. Effects of ACh applied as a bolus dose on tension of PE-precontracted *human* cavernous smooth muscle strips alone (*left*) and after inhibition (*right*) by L-NOARG

bromide, L-*N*-nitroarginine (L-NOARG) and tetrodotoxin (TTX) from Sigma; sodium nitroprusside (SNP) from Merck. The stock solution of L-NOARG at 2×10^{-2} mol/l was made in 40°C distilled water under sonification.

Experimental protocol

After an equilibraton period of 20 min in the bath chamber, tension of the strips was maintained (with adjustment as necessary) at 2 mN for 20 min. After another equilibration period of 60 min, strips were contracted with PE at 10^{-6} mol/l. Strips that reached a maximal tension of less than 10 mN were excluded from the study.

ACh was added either cumulatively (rabbit) or as a bolus dose (human), and isometric tension was recorded continuously. After washout and another 45 min of equilibration, atropine methylbromide and L-NOARG were added to PE-precontracted strips at 5×10^{-6} mol/l and 2×10^{-4} mol/l, respectively, at a stable tension. One strip from each rabbit was used for control experiments.

EFS was performed repeatedly: 50 V, 10 Hz; pulse duration, 1 ms; train duration, 10 s; train interval, 5–8 min. Immediately after the third stimulation. Atropine was added to the bath at 5×10^{-6} mol/l, and two more stimulations were performed. Finally, L-NOARG (2×10^{-4} mol/l) was added, and EFS was performed another three times. Effects of EFS were also measured after addition of TTX (5×10^{-7} mol/l) in six rabbit and six human strips. In six rabbit strips used as controls, responses to eight consecutively applied EFS pulses were monitored. At the end of each experiment, we induced relaxation with SNP (10^{-4} mol/l).

Statistics

Relaxation after ACh or EFS was measured as the difference between tension before ACh (or EFS) and minimal tension evoked by ACh or EFS. For each strip treated with ACh, the maximal relaxation induced by ACh at 3.16×10^{-5} mol/l (rabbit, cumulative) or at 10^{-5} mol/l (human, bolus) was taken as 100%. For each strip stimulated with EFS, the mean of three initial responses was taken as 100%. Relaxation in the presence of atropine and L-NOARG was expressed as a percentage of initial response plus or minus the standard error of the mean. *N* indicates the number or individuals used.

Statistical significance was tested with Wilcoxon's paired-rank test. The significant difference between relaxant effects in rabbit and human strips was tested with Wilcoxon's unpaired-rank test. Results with a P of less than 1% ($P \ll 0.01$) were significant.

Results

Effects of PE

PE caused stable contraction to 22.9 ± 9.7 mN ($N=38$) in rabbit CC and to 58.8 ± 15.6 mN ($N=10$) in human CC. (In previous experiments, application of PE at the same concentration was shown to cause about 40% of maximal PE-inducible tension in rabbit and human strips.) Time controls with PE showed a negligible decrease in tension of less than 10% in 30 min.

Effects of exogenously applied ACh

In rabbit CC, comulatively applied ACh up to 3×10^{-5} mol/l reduced PE-induced tension to 45.8 ± 7.8% of initial tension ($N=12$) (Fig. 1). Effects of ACh were slighly more pronounced when ACh was delivered as a bolus dose. Because availability was restricted, human strips were subjected to ACh only as a bolus dose; it reduced PE-induced tension to 50.1 ± 11.0% of initial tension ($N=6$) (Fig. 2). In both rabbit and human strips. ACh or L-NOARG abolished the effects of exogenously applied ACh. Addition of L-NOARG resulted in an increase in PE-induced tension to 129.1 ± 6.6% in rabbit ($N=6$) and to 122.9 ± 7.8% in human CC ($N=6$) (Figs. 1–3).

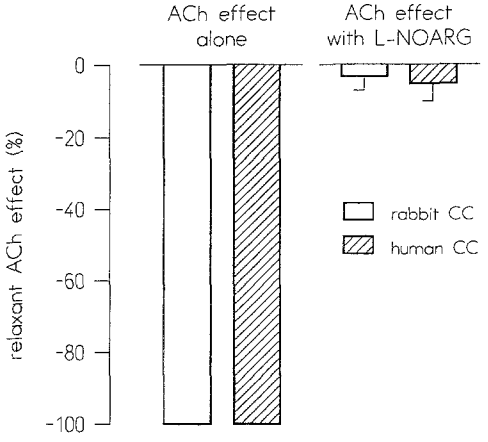

Fig. 3. Relaxant effects of ACh in rabbit ($N=6$) and human ($N=4$) cavernous smooth muscle strips alone and after inhibition by L-NOARG

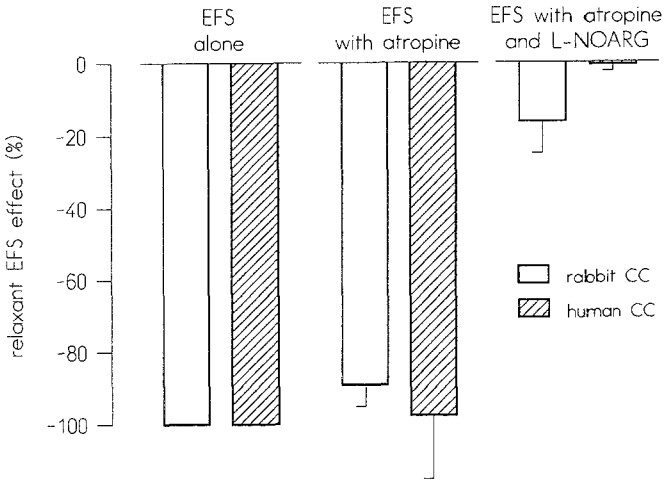

Fig. 5. Relaxant effects of EFS in rabbit ($N=12$) and human ($N=6$) cavernous smooth muscle strips precontracted by PE alone after inhibition by atropine and after application of atropine and L-NOARG

Effects of EFS

In rabbit strips, EFS induced transient relaxation to $37.3 \pm 24.9\%$ of initial tension ($N=38$). EFS relaxed human strips to $28.3 \pm 17.5\%$ of initial tension (Fig. 4). In the same strips, the relaxant effects of EFS remained unaltered even after eight applications of EFS ($N=6$). Occasionally, a small contraction preceded or followed relaxation. The effects of EFS in rabbit CC ($N=6$) and human CC ($N=6$) were abolished by TTX.

In the presence of atropine, the effects of EFS were reduced to $89.0 \pm 6.1\%$ in rabbit CC ($N=20$) and to $97.5 \pm 17.5\%$ in human strips ($N=6$) (Figs. 4, 5). L-NOARG significantly reduced the effects of EFS further to $16.2 \pm 8.7\%$ of the initial response in rabbit CC ($N=12$) and to $0.8 \pm 1.7\%$ in human CC ($N=6$). The differences

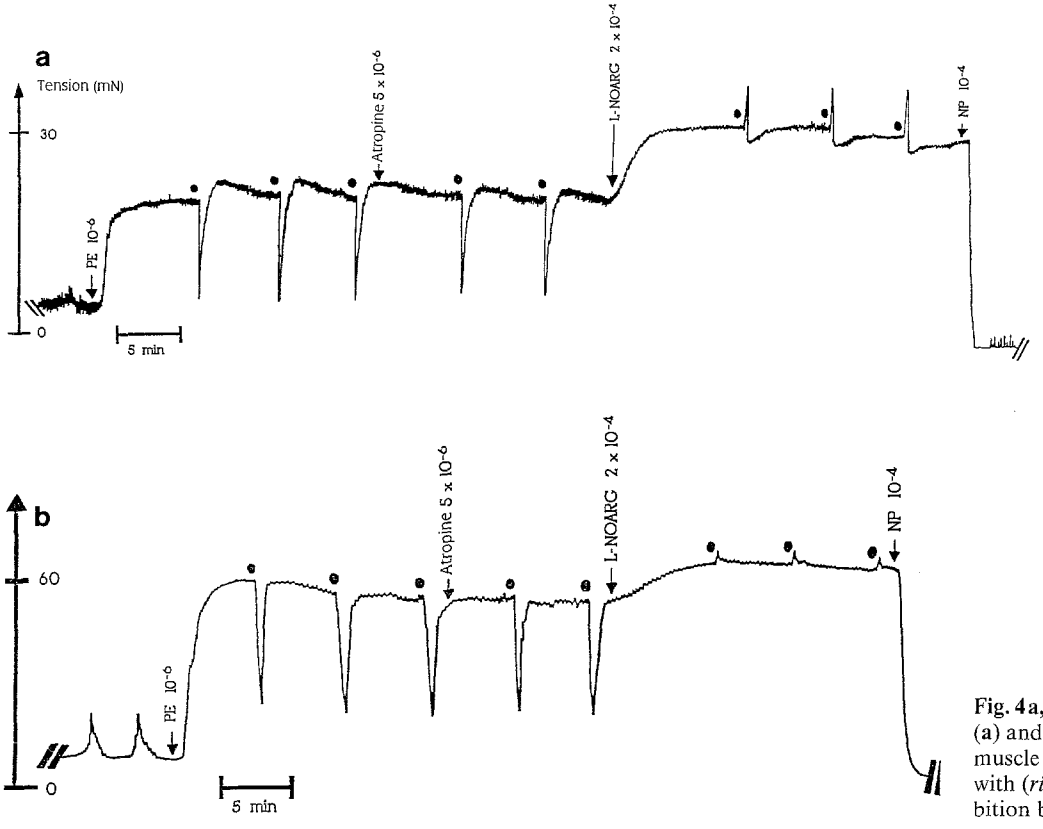

Fig. 4a, b. Effects of EFS on rabbit (**a**) and human (**b**) cavernous smooth muscle strips precontracted by PE with (*right*) and without (*left*) inhibition by L-NOARG

between species were also statistically significant (Figs. 4, 5). In strips obtained from diabetic men ($N=4$), the relaxant effects of ACh and EFS and the contraction after L-NOARG appeared to be significantly less than in CC taken from nondiabetic men ($N=6$).

Discussion

The tension of corpus cavernosum smooth muscle is generally thought to be controlled by various neurotransmitters and vascular endothelium that lines the sinusoidal spaces [21]. Relaxation of vascular smooth muscle induced by exogenously applied ACh has been shown to be mediated by endothelium [7, 21]. In some species, including rabbits and humans, the presence of cholinesterase-containing nerve fibers and cholinergic receptors in CC suggests ACh as the classical parasympathetic neurotransmitter in penile erection [24, 26].

Exogenously applied ACh reduced tension in PE-precontracted CC to about 50% of the initial tension. In previous experiments, the effects of ACh given as bolus doses, although slightly more pronounced, were not significantly different from after cumulative application. The similarity of the effects of ACh on human and rabbit CC in this study suggests that the mechanisms of relaxation in both species are based on the same principle. Abolition of the effects of ACh in both human and rabbit CC by atropine is evidence of the muscarinic mediation found by other authors [7, 21].

Stimulation of muscarinic receptors on endothelial cells causes release of so-called endothelium-derived relaxing factors [7]. Synthesis of NO, which has been identified as a powerful endothelium-derived relaxing factor [19], can be blocked by L-arginine analogues, such as L-NOARG [18, 20]. Addition of L-NOARG to PE-precontracted human and rabbit CC resulted in significant increases in tension. This might indicate that CC continuously generates NO, which controls smooth muscle tension, as already shown in basilar arteries [6] and in human subcutaneous resistance vessels [28]. Abolition of the effects of ACh in presence of L-NOARG is strong evidence that ACh-induced CC relaxation is mediated by NO. The blocking effects were similar in both human and rabbit CC.

The relaxant effects of EFS on rabbit and human CC were completely blocked by the sodium channel blocker TTX, thus proving mediation by neurotransmitters [27]. In the presence of functional adrenergic and cholinergic blockade, the relaxation of CC caused by EFS must be mediated by NANC neurotransmitters [11]. In our study, however, we compared the EFS effects with and without cholinergic inhibition by atropine, which allowed us to rule out muscarinic mediation as the main part of EFS-induced relaxation of CC strips in both human and rabbit. The endothelium as a diffusion barrier for atropine [15] is unlikely, because an increase in atropine concentration did not increase its minimal blocking effect in EFS-induced relaxation [13]. Therefore, cholinergic innervation of the endothelium is not likely, although like other authors [11] we failed to remove the endothelium sufficiently by rubbing the strips.

In contrast to atropine, further addition of L-NOARG reduced the effects of EFS in rabbit CC and reduced them even more in human CC. Although some other studies have failed to show the substantial role of NO during EFS stimulation [12, 23], our findings support the idea of NO formation as the main event in neurogenically induced relaxation of CC in rabbits and humans [9, 11]. The formation of NO, first shown in endothelial cells [19], has been demonstrated to account for smooth muscle relaxation, also in tissue free of endothelium [5, 8]. Furthermore, NO release in the brain has been shown and that suggests NO as a neurotransmitter [5, 8]. The hypothesis of NO as a peripheral NANC neurotransmitter has gained support with purification of the enzyme NO synthase [4] and immunohistological staining, which showed the presence of the enzyme in considerable amounts in the peripheral nervous structures [4]. Our findings in EFS-induced relaxation give strong evidence of NO as a relevant NANC transmitter in penile erection in rabbits and men. Our data concur with those of Aronson et al. [2], who additionally showed that a directly added NO compound induced relaxation in CC strips. Reduction of relaxant effects both after ACh and after EFS in tissue taken from diabetic impotent men might suggest the clinical importance of impaired NO formation as a cause of organic impotence [22]. Acting by an increase in cytosolic cAMP [14], peptidergic transmitters, such as VIP and CGRP and prostanoids, seem to play a minor part in the relaxation of CC, because NO effects are mediated by an increase in cGMP [12].

In conclusion, the results of our study show that cholinergic and neurogenic induction of corpus cavernosum smooth muscle relaxation with EFS is mediated mainly by NO. The relaxant effects of exogenous ACh might require the presence of intact endothelium, but neurogenically induced relaxation might be mediated by NO formation in neuronal tissue. Furthermore, there is strong evidence that a basal NO release controls cavernous smooth muscle tone. The fact that the effects were similar in rabbit CC and human CC suggests that the in vitro rabbit model is an appropriate tool for further investigation of NO effects. Clinical experiments with measurement of endothelium-dependent relaxation [3] and immunohistochemical studies on human cavernous tissue and the cavernous nerve with antisera to purified NO synthase will contribute further information on the role of NO in human penile erection.

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