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Effect of sildenafil on non-adrenergic non-cholinergic neurotransmission in bovine penile small arteries

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Abstract

The purpose of the present study was to investigate the effect of the phosphodiesterase isoenzyme V inhibitor, sildenafil, on non-adrenergic non-cholinergic neurogenic relaxations of intracavernous isolated penile small arteries. Dense plexes of nerve fibres immunoreactive for neural nitric oxide (NO) synthase were observed in the adventitia–media junction of the penile small arteries. In 5-hydroxytryptamine-contracted preparations, the inhibitor of NO synthase, N^G-nitro-L-arginine (L-NOARG), and of soluble guanylyl cyclase, 1*H*-[1,2,4]oxadiazolo[4,3,-*a*]quinoxalin-1-one (ODQ), reduced the electrical field stimulation-induced relaxations. Sildenafil and exogenous NO induced relaxations of penile small arteries. Sildenafil enhanced NO and vasoactive intestinal peptide-induced relaxations. Moreover, sildenafil increased the duration of the relaxations elicited by electrical field stimulation in penile small arteries and corpus cavernosum tissue. In the presence of L-NOARG, sildenafil only at supratherapeutic concentrations reduced the prazosin-sensitive contractions elicited by EFS in penile small arteries. Neurogenic NO-mediated and guanylyl cyclase-dependent relaxations of penile small arteries and corpus cavernosum tissue, considered to be associated with the vasodilatation leading to erection, are selectively enhanced by an inhibitor of phosphodiesterase V.

Keywords: Penile artery; Nerve; Electrical field stimulation; N^G-nitro-L-arginine; Nitric oxide (NO); VIP (vasoactive intestinal peptide); Sildenafil

1. Introduction

Penile erection is initiated by activation of parasympathetic pelvic nerves leading to arterial dilatation followed by relaxation of the corpora cavernosa. This allows filling of the sinusoids and entrapment of pressurized blood in the corpora cavernosa (Andersson and Wagner, 1995). Cholinergic nerves and vasoactive intestinal peptide (VIP) containing nerves present in penile tissues are thought to play a minor role, since penile erection is atropine-resistant (Brindley, 1986), and VIP alone causes relatively poor erections even after direct injection into the corpus cavernosum (Juenemann et al., 1987; Andersson and Wagner, 1995). Immunocytochemical demonstration of nitrergic nerves in the corpus cavernosum and penile vasculature, and the observations that nitric oxide (NO) synthase inhibitors antagonize penile erection induced by electrical stimulation in several animal species in vivo as well as non-adrenergic non-cholinergic (NANC) neurotransmission in isolated cavernous trabecular smooth muscle led to the suggestion that NO is the main neurotransmitter mediating penile erection (Ignarro et al., 1990; Andersson and Wagner, 1995; Burnett, 1997).

NO activates cytosolic guanylyl cyclase leading to increased cellular cyclic GMP (cGMP), which is associated with a reduction in intracellular calcium by mechanisms involving protein kinase G activation of Ca^{2+} pumps and Ca^{2+}-activated K^+ channels (Lincoln et al., 1996). cGMP is hydrolyzed by specific cyclic nucleotide phosphodiesterase enzymes (Polson and Strada, 1996). Intracavernous injection of papaverine has been widely used in the treatment of impotence (Montorsi et al., 1995), and is a non-selective inhibitor of the cellular phosphodiesterases leading to increased levels of intracellular cyclic AMP and to a lesser degree of cyclic GMP (Rüegg, 1992). Recently, a selective and potent inhibitor of type V cyclic GMP

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phosphodiesterase, sildenafil (Ballard et al., 1998), was found to have clinical efficacy in the treatment of male impotence following oral administration (Goldstein et al., 1998; Langtry and Markham, 1999). However, the effect of sildenafil has not been examined in the penile arteries.

In the bovine dorsal penile arteries that supply glans penis with blood, an inhibitory neurotransmission was described by Klinge and Sjöstrand (1974) and N^G-substituted analogues of L-arginine (Liu et al., 1991) and haemolysate (Bowman and Gillespie, 1983) were shown to inhibit the non-adrenergic non-cholinergic relaxations. Recently, we have also found both functional and histochemical evidence for a nitrergic innervation of horse and human penile small arteries which are important for the erection, since they control the blood flow between the systemic arterial circulation and the cavernous sinusoids (Simonsen et al., 1995, 1997a). In addition, it appeared that NO contributes in less extent to both the inhibitory neurotransmission and endothelium-dependent relaxations in the penile small arteries compared to the corpus cavernosum tissue (Simonsen et al., 1997a; Prieto et al., 1998). Therefore, the studies performed with sildenafil in corpus cavernosum tissue cannot necessarily be extrapolated to the penile circulation.

The purpose of the present study was to examine the effect of sildenafil on the NANC neurotransmission in bovine small penile arteries. The following procedures were applied: (i) The NO/L-arginine innervation was characterized by immunocytochemical studies for neural NO synthase (nNOS) and the effect of blockers of the pathway was examined. (ii) The effect of sildenafil was examined both on the NANC relaxations in contracted preparations and on the adrenergic contractions induced by electrical field stimulation. (iii) For comparison with the small arteries, the effect of sildenafil on corpus cavernosum tissue was also evaluated.

2. Materials and methods

2.1. Tissue preparation

Penises from young sexual mature bulls (18–24 months) were obtained at the local slaughterhouse, immediately after the animals were killed and placed in cold physiological salt solution (PSS) of the following composition (mM): NaCl 119, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $CaCl_2$ 2.5, ethylenediaminetetraacetic acid (EDTA) 0.027 and glucose 5.5. The solution was gassed with 5% CO_2 in 95% O_2 to maintain pH at 7.4.

2.2. NADPH diaphorase histochemistry and nitric oxide synthase immunohistochemistry

Samples of isolated proximal corpus cavernosum were immersed in cold (4°C) 4% paraformaldehyde in 0.1 M sodium phosphate-buffered saline (PBS, pH 7.3) for 24–48 h, and then placed in a cryoprotective PBS solution containing 30% sucrose for 24 h at 4°C. Sections in coronal plane (40 μm) were obtained using a freezing microtome and processed for NADPH-diaphorase histochemistry following the protocol of Vincent and Kimura (1992) with minor modifications. Briefly, the sections were incubated in a medium containing 1 g l^{-1} β-NADPH, 25 g l^{-1} nitroblue tetrazolium and 3% Triton X-100 in 0.1 M PBS (pH = 7.4), for 30–45 min at 37°C and protected from light. After incubation, the sections were rinsed in PBS, mounted, dehydrated in graded ethanol series, cleared with xylene and covered with DePeX. The following controls of the histochemical reaction were carried out: (1) incubation without the substrate β-NADPH, (2) incubation without the chromogen nitroblue tetrazolium in order to rule out possible non-specific formation of reaction products, and (3) overfixation of the tissue for 2 weeks in fixative medium. In all cases, no residual reaction was observed.

For immunohistochemistry, the free-floating sections were processed following the avidin–biotin–peroxidase method (ABC, Hsu and Raine, 1981). The sections were treated for 30 min with a mixture of 1% H_2O_2 and 90% methanol in distilled water for endogenous peroxidase inhibition in order to reduce the background reaction, and then washed in PBS several times. The following steps were performed: (1) the sections were incubated in normal goat serum (3%) and 0.2% Triton X-100 in PBS for 2 h, (2) then they were incubated for 48 h at 4°C in mouse anti-serum raised against neural NOS (Sigma clone no. NOS-B1) 1:2500 in the incubation solution, (3) the sections were reacted with a bionylated goat anti-mouse secondary serum (Chemicon) 1:400 for 2 h at room temperature, (4) incubated with the avidin–biotin complex (ABC) 1:100 for 90 min at room temperature, and (5) the immunocomplex was visualized with 0.05% 3,3-diaminobenzidine and 0.001% H_2O_2 in PBS. Sections were rinsed in several changes of PBS between steps. Rinsed sections were then mounted as described above for light microscopic examination. Omitting the NOS antibody eliminated NOS-immunoreactivity.

2.3. Dissection and mounting

The tunica albuginea of the proximal part of the penis was opened and corpus cavernosum was removed and placed in a dissection dish containing cold PSS, 4°C. The profound artery, orientated parallel to the longitudinal axis corresponding to the deep penile artery in man was localized, and second to third order branches were dissected by carefully removing the adhering trabecular tissue. Segments (ca. 2 mm long) of the small vessels were subsequently mounted as ring preparations on two 40 μm wires in microvascular double myographs by fixing one of the wires to a force transducer for isometric tension recording

and the second wire to a length displacement device as earlier described (Simonsen et al., 1997a,b,c).

The vessels were allowed to equilibrate in PSS, 37°C, pH 7.4 for about 30 min. The relation between resting wall tension and internal circumference was determined, and from this the internal circumference L_{100} corresponding to a transmural pressure of 100 mm Hg for a relaxed vessel in situ was calculated. The vessels were set to the internal circumference L_1, given by $L_1 = 0.9L_{100}$. Preliminary experiments showed that the force development is close to maximal at this internal circumference. The effective internal lumen diameter was determined as $l_1 = L_1/\pi$.

Corpus cavernosum smooth muscle strips with a size of $2 \times 2 \times 7$ mm were tied at each end with cotton threads and mounted in 5 ml capacity organ chambers with one end connected to an isometric force transducer and the other to a micrometer device. The resting tension was set to 50–55 mN and the tissue was allowed to equilibrate in PSS.

2.4. Experimental protocols in penile small arteries

The vessels were stimulated twice with 5-hydroxytryptamine (5-HT, 10^{-5} M) and the second time it was relaxed with the endothelium-dependent vasodilator acetylcholine (10^{-5} M). Then the endothelial cells were removed by introducing a human scalp hair through the vessel lumen and gently pushing the hair back and forth. The absence of endothelium was assessed by exposure to acetylcholine (10^{-5} M), in 5-HT contracted vessels. The arteries were incubated with guanethidine (10^{-5} M) and atropine (10^{-7} M) and these drugs were kept present throughout the rest of the experiment to block adrenergic neurotransmission and muscarinic receptors, respectively.

Electrical field stimulation was performed with platinum electrodes (J.P. Trading, Aarhus, Denmark), measuring 2×2 mm, which were secured in plastic mounting heads on either side of the artery, approximately 1 mm from the vessel wall. The electrodes were connected to an electrical stimulator (Cibertec CS20, Barcelona, Spain) with constant current output adjusted to 35 mA. The arteries were contracted to 5-HT (10^{-6} M), and when a stable contraction level was attained, a frequency–response curve (1–32 Hz) was constructed. The bath volume was changed several times and the vessel was allowed to equilibrate, before it was incubated for 30 min with an inhibitor of NO synthase, N^G-nitro-L-arginine (L-NOARG, 3×10^{-5} M), an inhibitor of guanylyl cyclase, 1*H*-[1,2,4]oxadiazolo[4,3,-*a*]quinoxalin-1-one (ODQ, 3×10^{-6} M), or tetrodotoxin (10^{-6} M), and a second frequency–response curve was performed in the presence of the treatment. In each experiment control frequency–response curves without any treatment were run in parallel. Finally, the effect of ODQ was evaluated on the concentration relaxation curves for *S*-nitroso-*N*-acetylpencillamine. Control responses were obtained in the parallel control vessel from the same animal. At the end of each series of experiments, papaverine was added in a concentration of 10^{-4} M to attain maximum relaxation of the arteries.

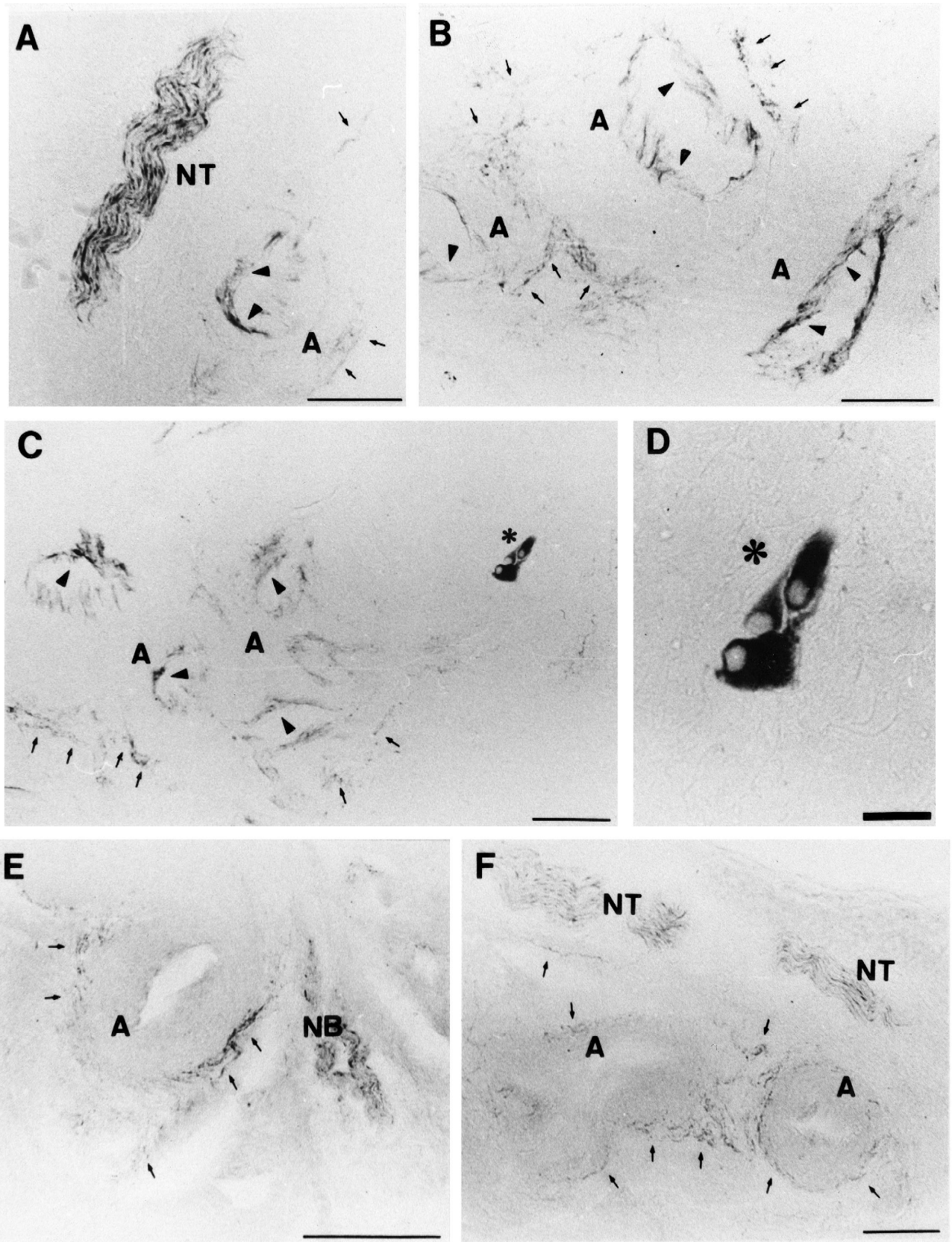
The effect of sildenafil on non-adrenergic non-cholinergic relaxations induced by electrical field stimulation were evaluated as earlier described for the α_2-adrenoceptor antagonist, rauwolscine (Simonsen et al., 1997c). Endothelium-intact preparations were incubated with guanethidine (10^{-5} M) and atropine (10^{-7} M), and activated with 5-HT (10^{-6} M), and when a stable contraction was attained, the vessel was stimulated with 1, 4 and 32 Hz. The arteries were repeatedly washed and allowed to equilibrate for at least 30 min, before they were contracted with 5-HT (10^{-6} M) and when a stable contraction was attained, experiments were performed in the presence of sildenafil (10^{-9}, 3×10^{-9}, and 10^{-8} M). If sildenafil decreased the 5-HT-contraction below 50%, additional 5-HT was added until a response comparable to that in the first exposure was obtained, and when this contraction was stable, the preparation was stimulated with electrical field stimulation. Parallel time control response to electrical field stimulation in the absence of drug was also constructed. Finally, the vessel was incubated with either L-NOARG (3×10^{-5} M) or tetrodotoxin (10^{-6} M) and the electrical field stimulation was repeated.

In order to evaluate the effect of sildenafil on relaxations induced by exogenous NO added as acidified sodium nitrite ($NaNO_2$), a first concentration–response curve was constructed. The preparations were washed and allowed to equilibrate. Then they were contracted with 5-HT (10^{-6} M) and a second concentration–response curve for NO was obtained in the presence of sildenafil (10^{-8} M). A parallel concentration–response curve for NO obtained in a vascular segment from the same animal served as control. The effect of sildenafil was also evaluated on the relaxations induced by vasoactive intestinal peptide (VIP). Two consecutive segments of penile arteries were mounted as described above and a concentration–response curve was constructed for VIP (10^{-10}–3×10^{-7} M) in the absence and the presence of sildenafil.

In 5-HT-contracted penile arteries acetylcholine and sildenafil relaxations were characterized. Two consecutive segments of penile artery from the same animal were mounted and concentration–response curves were constructed for acetylcholine and sildenafil in endothelium-denuded and intact preparations in the absence or presence of L-NOARG (3×10^{-5} M), L-NOARG (3×10^{-4} M), or the combination of L-NOARG (3×10^{-4} M) and indomethacin (3×10^{-6} M).

To examine whether sildenafil inhibit the adrenergic contractions in penile small arteries, the preparations were incubated with L-NOARG (3×10^{-5} M) and propranolol (10^{-6} M) to inhibit the concomitant NO release and β-adrenoceptors, respectively, when the preparations are activated with noradrenaline or electrical field stimulation (Simonsen et al., 1997b). Preparations were either acti-

vated with noradrenaline (10^{-6} M) or 16 Hz electrical field stimulation (supramaximal current) applied with 3-min intervals, and increasing concentrations of sildenafil or prazosin were added. Control responses in the absence of



treatment were obtained in a vascular segment examined in parallel.

2.5. Experimental protocols in corpus cavernosum tissue

The tissue was activated by adding potassium physiological salt solution (KPSS, equivalent to PSS but NaCl replaced by KCl on an equimolar basis, giving a final concentration of 123.7 mM K^+), which also stabilized the subsequent submaximal contractile responses to phenylephrine (10^{-5} M) and 5-HT (3×10^{-6} M). The strips were incubated with guanethidine and atropine to block the adrenergic neurotransmission and the muscarinic receptors, respectively. Control relaxations for 1, 4, 16, and 32 Hz (1 ms, 20 s trains, 120 mA) was obtained in preparations activated with either 5-HT (3×10^{-6} M) or phenylephrine (10^{-5} M), which induced contractions corresponding to 70% of the response to KPSS. A second frequency response relationship was obtained either in the absence or the presence of sildenafil, L-NOARG, or tetrodotoxin (10^{-6} M).

2.6. Drugs

The following drugs were used: sildenafil citrate (a gift from Pfizer Central Research Center, Sandwich, England); acetylcholine chloride, atropine sulphate, guanethidine, 5-hydroxytryptamine (5-HT, serotonin), indomethacin, (−)-noradrenaline hydrochloride, N^G-nitro-L-arginine, papaverine hydrochloride, phenylephrine hydrochloride, prazosin hydrochloride, propranolol hydrochloride, synthetic vasoactive intestinal peptide (VIP, human, porcine, rat), sodium nitrite ($NaNO_2$), and tetrodotoxin were from Sigma (St. Louis, MO, USA); 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ) and *S*-nitroso-*N*-acetylpencillamine were purchased from Tocris Cookson (UK); All drugs were dissolved in twice distilled water, except indomethacin and ODQ, which were dissolved in 90% ethanol and dimethyl sulphoxide, respectively, and further diluted in water. These drugs were added in volumes not exceeding 0.3% of the tissue bath (10 ml). Stock solutions were prepared and stored at −20°C and further fresh solutions were prepared daily. Stock solutions of guanethidine, atropine and sildenafil were prepared for each experiment.

The $NaNO_2$ was freshly prepared as 1 M stock solutions by adjusting the pH to 2 by adding concentrated HCl as earlier described (Simonsen et al., 1995). The stock solution was kept cold and protected from air. Further dilutions were made in diluted HCl (pH 2) immediately before use and added in volumes of 5–10 μl. Previous experiments showed that the acid vehicle had no effect on the preparations at the final concentration applied.

2.7. Analysis of data

Mechanical responses of the arteries were measured as force and expressed as active wall tension, ΔT (N m^{-1}), which is the increase in force, ΔF (mN), divided by twice the segment length. The responses of corpus cavernosum strips are expressed as increase in force ΔF (mN). The magnitude of relaxant responses is given as percentage of the preconstriction level just prior to addition of the agonist. Duration of the relaxations induced electrical field stimulation and acidified $NaNO_2$ was measured as time from 50% relaxation to 50% recovery of tone. VIP induced sustained relaxations and duration was measured from the initiation of relaxation to 50% relaxation.

By using a computer program (GraphPad, Institute for Scientific Information, San Diego, CA, USA), the concentration–response curves to the different relaxant agents were fitted to the classical "Hill-equation": $R/R_{max} = A(M)^n/(A(M)^n + EC_{50}(M)^n)$, where R/R_{max} is the relative response to the effective concentration of drug, $A(M)$, and $EC_{50}(M)$ is the concentration of agonist required to give half maximal inhibition (R_{max}) of the precontraction, when $A(M)$ and $EC_{50}(M)$ are given in molar concentrations. n is a curve fitting parameter or "Hill-coefficient". EC_{50} values are expressed as the negative log molar concentration, $pD_2 = -\log(EC_{50})$.

Results are expressed as means ± S.E.M. and n represents the number of arteries (one from each animal). Statistical differences between means were determined by Student's t test for paired observations. Means of multiple groups were compared by one-way analysis of variance (ANOVA) and Bonferroni method as a post test. Probability levels under 5% were considered significant.

3. Results

3.1. NADPH-diaphorase histochemistry and immunohistochemistry

Neural NO synthase-containing fibres were localized in the corpus cavernosum of the bull by NADPH-diaphorase

Fig. 1. Nitrergic nerves in bovine erectile tissue. NADPH-d activity (A–D) and neural nitric oxide synthase (nNOS)-immunoreactivity (E–F) in sections of the bovine corpus cavernosum. (A) Thick nerve trunk (NT) close to a muscular artery (A) surrounded by a weak plexus of positive nerves (arrows). A positive reaction was present in the arterial endothelium (arrowheads). (B) Group of helicine arteries (A) showing a plexus of positive nerves (arrows) and positive reaction in endothelium (arrowheads). (C) General view in the corpus cavernosum with a group of arteries (A) surrounded by a plexus of positive nerves at the adventitia (arrows) and positive reaction in the endothelium (arrowheads). Positive nerve cell bodies are observed within an intramural ganglia (asterisk). (D) Detail of (C) showing the ganglia with the positive nerve cell bodies in the corpus cavernosum. (E) A nerve bundle of NOS immunoreactive fibres (NB) forming a plexus of nerves (arrows) around the artery (A). (F) Different nerve trunks (NT) close to arteries (A) and nerve fibres in the cavernosum tissue or forming plexus around the artery (arrows). Scale bar: A, B, C, E, and F 100 μm; D 25 μm.

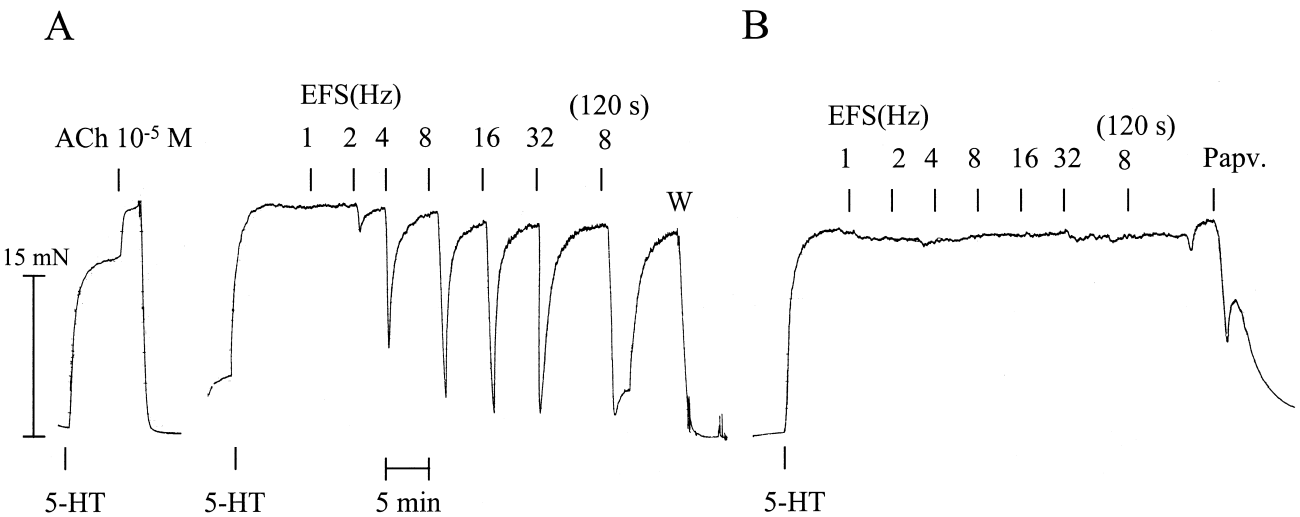


Fig. 2. Non-adrenergic non-cholinergic neurogenic relaxations in penile arteries. Isometric force recordings showing (A) that electrical field stimulation (EFS) with square pulses (0.3 ms, 20 s trains, supramaximal current) elicited frequency-dependent (1–32 Hz) relaxations of endothelium-denuded bovine intracavernous small penile arteries treated with 10^{-5} M guanethidine and 10^{-7} M atropine to block adrenergic and cholinergic neurotransmission, respectively, and contracted with 5×10^{-7} M 5-hydroxytryptamine (5-HT). (B) Electrical field stimulation performed in the presence of tetrodotoxin. Papv.: papaverine 10^{-4} M. W: wash.

histochemistry or by immunohistochemistry by using an antibody specific for nNOS (Fig. 1). Both approaches revealed the presence of fibres with different thickness and form in the corpus cavernosum, and the results with the two methods were almost similar. The only exception was the presence of NADPH-diaphorase activity in the endothelial cells (Fig. 1A–C). The positive fibres penetrated the tunica albuginea together with the vascularization for the corpus cavernosum. The distribution of the innervation in the corpus cavernosum was heterogenous. Thus, nerve trunks and bundles of fibres were observed in some zones while in other zones there was no presence of NADPH-d positive fibres (Fig. 1C). In addition to NADPH-d positive fibres, a small number of intramural ganglia that were dispersed in the corpus cavernosum were found (Fig. 1C). These ganglia contained 1–3 neurons (Fig. 1D). The muscular arteries of different size were surrounded by a fine plexus of NADPH-d positive and nNOS-ir fibres in the adventitia (Fig. 1E–F).

3.2. Non-adrenergic non-cholinergic neurotransmission in penile small arteries

5-HT induced concentration-dependent contractions in endothelium-intact bovine penile arteries with pD_2 values and maximal responses of 6.94 ± 0.05 and 7.9 ± 0.9 N

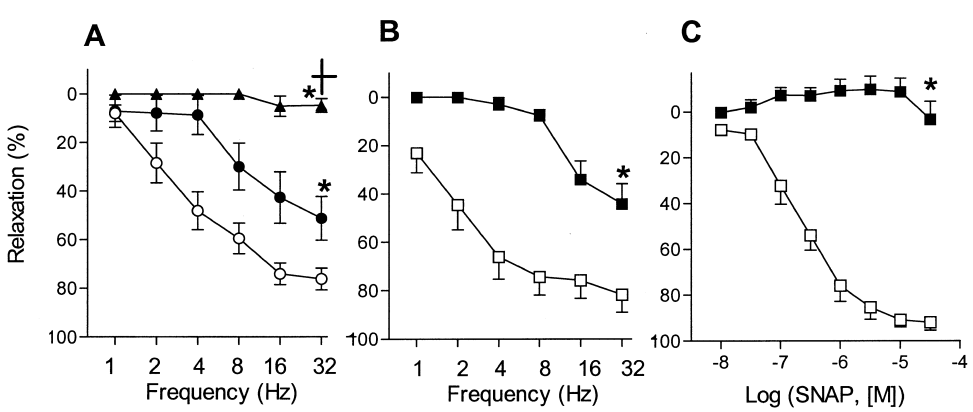


Fig. 3. NO-mediated neurogenic relaxations in penile arteries. Average relaxations to electrical field stimulation (0.3 ms, 20 s trains, supramaximal current) in bovine small penile arteries contracted with 5-hydroxytryptamine (5-HT, 5×10^{-7} M) in the presence of guanethidine and atropine under control conditions (open circles), and (A) the effect of L-NOARG (3×10^{-5} M, closed circles) and tetrodotoxin (10^{-6} M, closed triangles). Relaxations induced by (B) electrical field stimulation and (C) SNAP in endothelium-denuded bovine small penile arteries in the absence (open squares) and presence (closed squares) of the guanylyl cyclase inhibitor, ODQ (3×10^{-6} M). Relaxations are expressed as percentages of the tension induced by 5-hydroxytryptamine (5×10^{-7} M), and each point represents the mean ± S.E.M. of 5–8 endothelium-denuded arteries (one per animal). Significantly different curve maximum, tested by analysis of variance followed by Bonferroni method: $^{*}P < 0.05$, vs. control curve; †$P < 0.05$ vs. curve performed in the presence of L-NOARG.

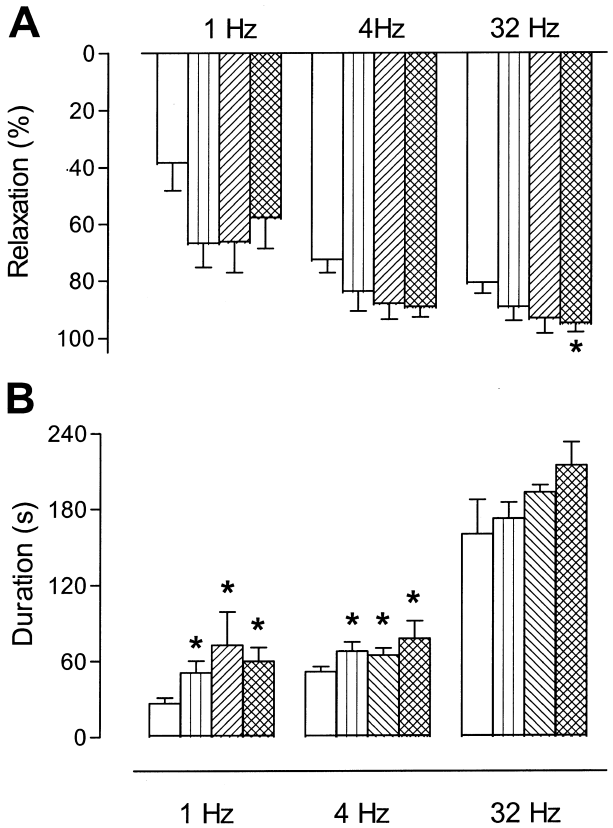


Fig. 4. Sildenafil enhances non-adrenergic non-cholinergic neurogenic relaxations. Effect of the selective inhibitor of phosphodiesterase V, sildenafil, on neurogenic relaxations in penile small endothelium-intact arteries. (A) Average relaxations and (B) duration of the relaxations induced by electrical field stimulation (EFS) applied with increasing frequency in the presence of vehicle (open bars) and the presence of 10^{-9} M (vertical line bars), 3×10^{-9} M (hatched bars), or 10^{-8} M (crossed bars) sildenafil. Results represent mean and vertical bars S.E.M. of 6–7 preparations. Significantly different parameter tested by analysis of variance followed by Bonferroni method: $^{*}P < 0.05$, vs. vehicle control curve.

m^{-1} ($n = 9$), respectively. Endothelial cell removal caused small, although not significant, leftward shifts in the concentration–response curves for 5-HT ($n = 6$). Therefore, to obtain comparable precontractions corresponding to 60–70% of the maximum response, 10^{-6} M and 5×10^{-7} M 5-HT were applied, respectively, in endothelium-intact and denuded segments. The 5-HT-induced contractions were stable for a prolonged period of 40–60 min ($n = 12$).

In 5-HT (5×10^{-7} M)-contracted endothelium-denuded arteries treated with guanethidine and atropine, electrical field stimulation (0.3 ms, 20 s trains, supramaximal current) caused frequency-dependent (1–32 Hz) relaxations of bovine penile arteries with an $EF_{50} = 3.6 \pm 0.3$ Hz and maximum relaxation of $82 \pm 2\%$ ($n = 41$, Fig. 2A). These relaxations were reproducible and in control experiments run parallel to the drug treatments described below, electrical field stimulation induced relaxations with EF_{50}-values and maximum relaxation of respectively, 3.7 ± 0.7 Hz and $80 \pm 4\%$ ($n = 13$) in a second frequency–response curve. These relaxations were abolished in the presence of 10^{-6} M tetrodotoxin ($n = 6$, Figs. 2B and 3A).

In endothelium-denuded segments, the NOS inhibitor, L-NOARG (3×10^{-5} M), or the inhibitor of guanylyl cyclase, ODQ (3×10^{-6} M) had no effect on resting tension, but they inhibited the electrical field stimulation-induced relaxations at the lowest frequencies (1–4 Hz) and attenuated the responses at higher frequencies (8–32 Hz) (Fig. 3A,B). The NO donor, *S*-nitroso-*N*-acetylpencillamine, induced potent relaxations of 5-hydroxytryptamine-contracted preparations with pD_2-values and maximal relaxations of 6.46 ± 0.23 and $92 \pm 3\%$ ($n = 5$), respectively. In the presence of ODQ (3×10^{-6} M), *S*-nitroso-*N*-acetylpencillamine did not cause relaxation of the penile small arteries (Fig. 3C).

In endothelium-denuded arteries, the VIP receptor antagonists, VIP-(6–28) (3×10^{-7} M) and VIP-(10–28) (3×10^{-6} M) had no effect on concentration–response curves for VIP or the frequency–response curves for electrical

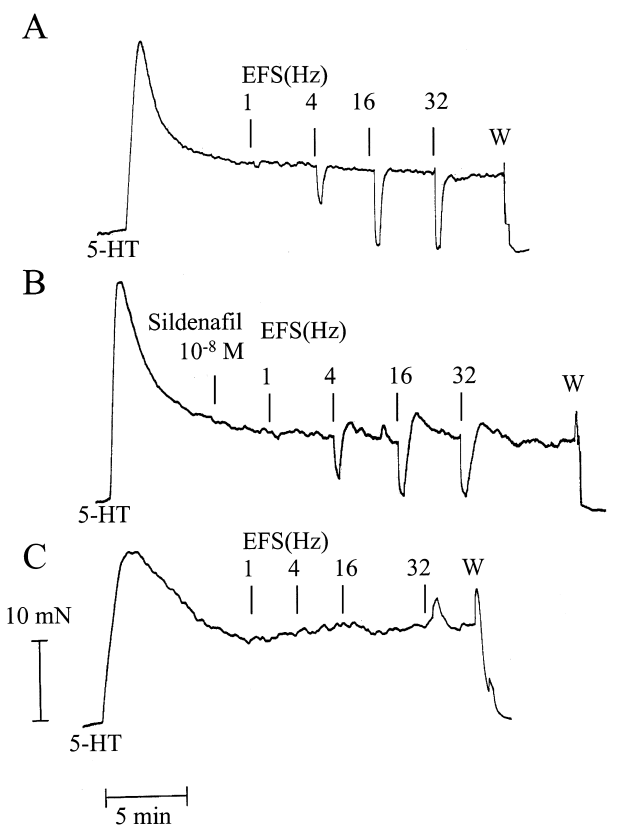


Fig. 5. Sildenafil enhances the duration of NO-mediated neurogenic relaxations of corpus cavernosum tissue. Isometric force recordings showing (A) that electrical field stimulation (EFS, 1 ms, 20 s trains) elicited frequency-dependent relaxations of 5-hydroxytryptamine (5-HT, 3×10^{-6} M)-contracted bovine corpus cavernosum strips treated with 10^{-5} M guanethidine and 10^{-7} M atropine. (B) EFS performed in the presence of sildenafil (10^{-8} M). (C) EFS performed in the presence of L-NOARG (3×10^{-5} M). W, wash.

field stimulation obtained in the absence or presence of L-NOARG (3×10^{-5} M) ($n = 6$ for each protocol, results not shown).

3.3. Effect of sildenafil on the non-adrenergic non-cholinergic neurogenic relaxations in penile small arteries and corpus cavernosum

In endothelium-intact penile small arteries treated with guanethidine and atropine, and contracted with 5-HT (10^{-6} M), electrical field stimulation caused relaxation (Fig. 4A). When the responses were repeated three times, contractions induced by 5-HT and relaxations induced by electrical field stimulation were reproducible. Sildenafil lowered the contraction induced by 5-HT and sometimes additional 5-HT, up to 5×10^{-6} M, had to be added to obtain comparable preconstrictions. Thus, 5-HT contracted the penile arteries 2.7 ± 0.3 N m^{-1} ($n = 17$) in the absence of drug, and 2.3 ± 0.4 N m^{-1} ($n = 7$), 2.1 ± 0.5 N m^{-1} ($n = 7$), and 2.2 ± 0.4 N m^{-1} ($n = 6$), respectively, in the presence of 10^{-9}, 3×10^{-9}, and 10^{-8} M sildenafil. Compared to the parallel responses obtained in the presence of vehicle, sildenafil increased significantly the duration of the relaxations induced by the lowest frequencies (1 and 4 Hz) of electrical field stimulation and there was only an effect on the magnitude of the relaxations at 32 Hz stimulation (Fig. 4A,B).

125 mM KPSS increased force in the corpus cavernosum strips 16.2 ± 1.0 mN ($n = 20$). 5-HT (3×10^{-6} M) and phenylephrine (10^{-5} M) induced contractions of 10.6 ± 1.0 and 13.5 ± 1.0 mN, respectively, corresponding to 65–80% of the KPSS response.

In 5-HT-contracted corpus cavernosum tissue, sildenafil (10^{-10}–3×10^{-6} M) caused concentration-dependent relaxations with pD_2 and maximal relaxations of 7.48 ± 0.2 and $53 \pm 4\%$ ($n = 5$), although these relaxations were less pronounced compared to penile small arteries. The sildenafil-induced relaxations were abolished in the presence of ODQ (3×10^{-6} M, $n = 4$).

In 5-HT- and phenylephrine-contracted endothelium-intact preparations treated with guanethidine and atropine, electrical field stimulation caused relaxations (Fig. 5A) which were abolished in the presence of tetrodotoxin (10^{-6} M, $n = 4$). Sildenafil (10^{-8} M) enhanced the duration of the relaxations induced by electrical field stimulation in both 5-HT- and phenylephrine-contracted preparations (Figs. 5B and 6B,D). The effect of sildenafil (10^{-8} M) on the magnitude of relaxations was only modest, since it increased the magnitude of the relaxations induced by electrical field stimulation at the lowest frequencies applied and only in phenylephrine-contracted preparations (Fig. 6A,C). L-NOARG (3×10^{-5} M) abolished the electrical field stimulation-induced relaxations of corpus cavernosum tissue ($n = 5$, Fig. 5C).

NO, added as acidified $NaNO_2$, relaxed 5-HT-contracted endothelium-denuded penile arteries with pD_2 values and maximal relaxations of respectively 5.34 ± 0.15 and $98 \pm 1\%$ ($n = 5$, Fig. 7A). The concentration–re-

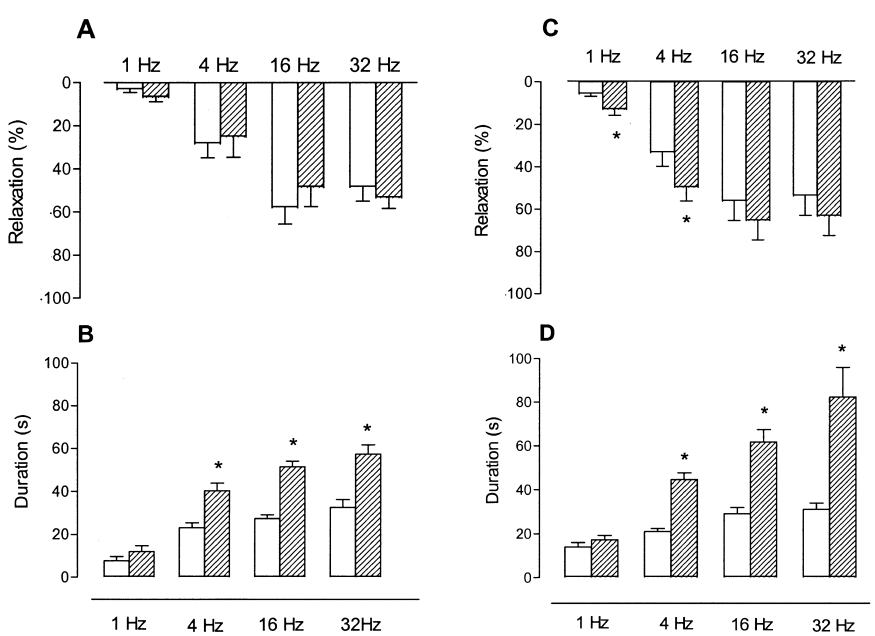


Fig. 6. Sildenafil enhances duration of NO-mediated neurogenic relaxations in corpus cavernosum tissue independent of the preconstrictor applied. Average (A–C) relaxations and (B–D) duration of relaxations induced by electrical field stimulation in the absence (open columns) and the presence (hatched columns) of 10^{-8} M sildenafil in corpus cavernosum strips contracted with either (A–B) 5-hydroxytryptamine (3×10^{-6} M) or (C–D) phenylephrine (10^{-5} M). Results represent mean and vertical bars S.E.M. of 7–8 preparations. $^{*}P < 0.05$, significantly different parameter compared to the parallel vehicle control.

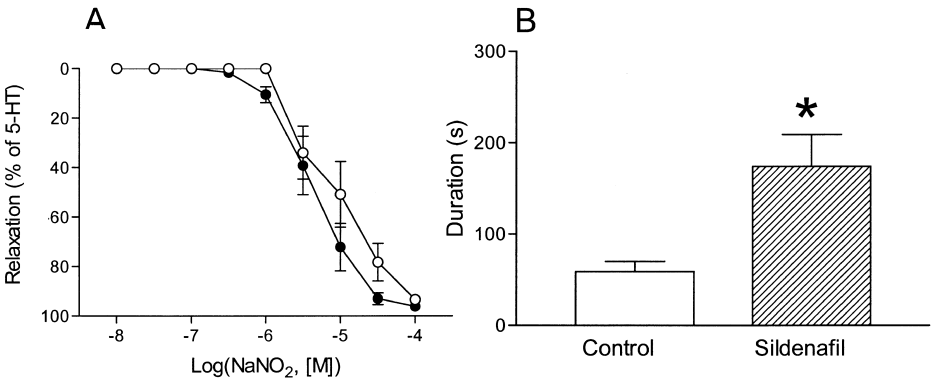


Fig. 7. Sildenafil enhances duration of NO-induced relaxations in penile small arteries. (A) Average concentration–response curves for NO, added as acidified $NaNO_2$, in the absence (open circles) and the presence of 10^{-8} M sildenafil (closed circles) in endothelium-denuded arterial segments contracted with 5-hydroxytryptamine (5-HT). (B) Duration of relaxation induced by 3×10^{-7} M NO in the absence (open column) and the presence (hatched column) of 10^{-8} M sildenafil. Results represent mean and vertical bars S.E.M. of 5–7 preparations.

sponse curves for NO were unchanged in the presence of sildenafil (10^{-8} M), but sildenafil increased the duration of relaxation induced by 10^{-7} M NO (Fig. 7B).

In 5-HT (10^{-6} M)-contracted endothelium-intact penile small arteries, VIP (10^{-10}–3×10^{-7} M) induced concentration-dependent relaxations with pD_2-values and maximal relaxations of 8.32 ± 0.18 and $88 \pm 3\%$ ($n = 18$). Endothelial cell removal or incubation with L-NOARG (3×10^{-5} M) evoked rightward shifts in the concentration–response curves for VIP in the penile arteries (Fig. 8A). Incubation with ODQ did inhibit the relaxations caused by the lowest concentrations of VIP while the maximal relaxations were unchanged (Fig. 8B). In endothelium-intact segments sildenafil (10^{-9} M) caused left-

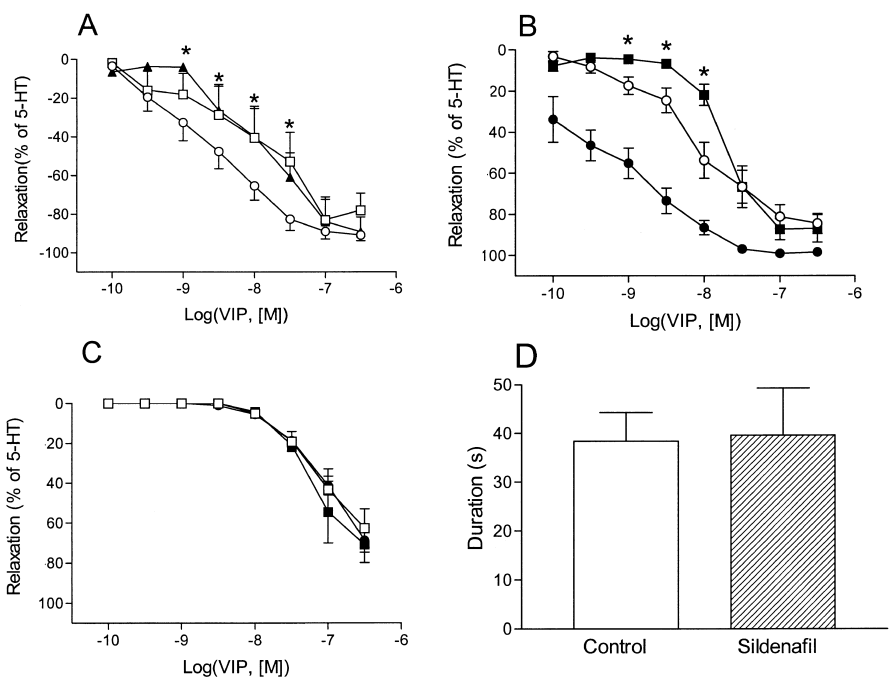


Fig. 8. VIP-induced relaxation and effect of sildenafil in penile arteries. (A) Average concentration–relaxation curves for VIP in endothelium-denuded arteries (open squares), and endothelium-intact preparations in the absence (open circles) and the presence of L-NOARG (3×10^{-5} M, closed triangles). (B) Average concentration–relaxation curves for VIP in endothelium-intact arteries contracted with 5-hydroxytryptamine (10^{-6} M) in the absence (open circles) and the presence of ODQ (3×10^{-6} M, closed squares) or sildenafil (10^{-9} M, closed circles). (C) Average concentration–relaxation curves for VIP in endothelium-denuded arteries contracted with 5-hydroxytryptamine (5×10^{-7} M) in the absence (open squares) and the presence of ODQ (3×10^{-6} M, closed squares) or sildenafil (10^{-8} M, closed circles). (D) Duration of relaxation induced in endothelium-denuded penile arteries induced by 10^{-7} M VIP in the absence (open columns) and the presence (hatched columns) of 10^{-8} M sildenafil. Points represent mean and vertical bars S.E.M. of 5–7 preparations. Significantly different responses compared to the parallel control curve: $^{*}P < 0.05$.

ward shifts of the concentration–response curves for VIP (Fig. 8B). In contrast, in endothelium-denuded arterial segments incubation with either ODQ (3×10^{-6} M) or sildenafil (10^{-8} M) did not change the concentration–response curves for VIP, and sildenafil neither changed how fast VIP (10^{-7} M) evoked relaxation (Fig. 8C–D).

3.4. Vasorelaxant effect of sildenafil in 5-HT-contracted penile arteries

In endothelium-intact penile small arteries contracted with 5-HT (10^{-6} M), the selective inhibitor of phosphodiesterase V, sildenafil, induced potent relaxations with pD_2 values and maximal relaxations of 8.39 ± 0.08 and $86 \pm 3\%$ ($n = 19$). The sildenafil-induced relaxations were abolished in the presence of ODQ (3×10^{-6} M) ($n = 6$). Endothelial cell removal abolished the relaxations induced by acetylcholine and caused significant inhibition of sildenafil relaxation (Fig. 9). In endothelium-intact arteries incubation with L-NOARG did not change the concentration–response curves for sildenafil, and L-NOARG caused only modest inhibition of the concentration–response curves for acetylcholine (Fig. 9). In contrast, incubation with both L-NOARG and the cyclooxygenase inhibitor, indomethacin, evoked significant rightward shifts in the concentration–response curves and inhibited the maximal responses for both sildenafil and acetylcholine (Fig. 9).

3.5. Effect of sildenafil on adrenergic contractions in penile small arteries

In penile small arteries incubated with L-NOARG and propranolol (10^{-6} M), noradrenaline (10^{-6} M) and electrical field stimulation 16 Hz induced contractions of 3.2 ± 0.5 N m^{-1} ($n = 11$) and 1.3 ± 0.2 N m^{-1} ($n = 18$), respectively. Sildenafil (10^{-10}–10^{-5} M) did not have a significant relaxant effect in noradrenaline-contracted penile arteries, but papaverine 10^{-4} M relaxed the same arteries $93 \pm 2\%$ ($n = 6$). Sildenafil only inhibited the 16 Hz electrical field stimulation-induced contractions with $54 \pm 4\%$ ($n = 6$) at the highest concentration (10^{-5} M) applied (Fig. 10). In contrast, the α_1-adrenoceptor antagonist, prazosin caused potent relaxations in noradrenaline-contracted preparations and inhibited the electrical field stimulation-induced contractions (Fig. 10). Tetrodotoxin abolished the electrical field stimulation-induced contractions ($n = 5$).

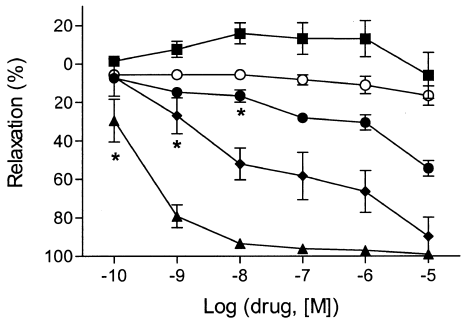


Fig. 10. Lack of inhibitory effect of sildenafil on adrenergic contractions of penile small arteries. Endothelium-intact small arteries were either contracted with noradrenaline (NA, 10^{-6} M) and relaxed with increasing concentrations of either sildenafil (closed squares) or the α_1-adrenoceptor antagonist, prazosin (closed triangles) or contracted with electrical field stimulation (EFS, 16 Hz, 0.3 ms, 20 s trains each 4 min) in the absence (open circles) or the presence of sildenafil (closed circles) or prazosin (closed diamonds). The experiments were performed in the presence of the NO synthase inhibitor, N^G-nitro-L-arginine, L-NOARG (3×10^{-5} M), and the β-adrenoceptor antagonist, propranolol (10^{-6} M). Results represent mean and vertical bars S.E.M. of 5–6 preparations. First concentration of drug causing significant relaxation: $^*P < 0.05$.

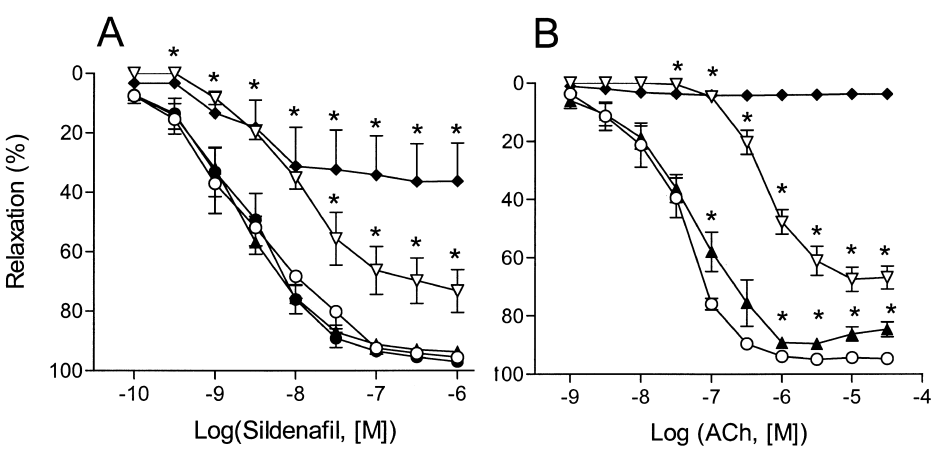


Fig. 9. Characterization of sildenafil and acetylcholine relaxation in 5-hydroxytryptamine-contracted penile arteries. Average concentration–response curves for (A) sildenafil and (B) acetylcholine (ACh) in endothelium-denuded segments (closed diamonds) and endothelium-intact penile arteries contracted with 5-hydroxytryptamine in the absence (open circles) and the presence of L-NOARG (3×10^{-5} M, closed circles), L-NOARG (3×10^{-4} M, closed triangles), or the combination of L-NOARG (3×10^{-4} M) and indomethacin (3×10^{-6} M, open triangles). Results represent mean and vertical bars S.E.M. of 5–6 preparations. Significantly different responses compared to the parallel control curve: $^*P < 0.05$.

4. Discussion

The main objective of the present study was to investigate the effect of sildenafil on NANC relaxations in penile small arteries. The findings give both functional and histochemical evidence for nitrergic innervation of bovine intracavernous arteries. The selective inhibitor of phosphodiesterase V, sildenafil, enhances mainly the duration of non-adrenergic non-cholinergic relaxations induced by electrical field stimulation in penile small arteries and smooth muscle strips of corpus cavernosum. The effect of sildenafil appears to be selective for the NANC relaxations, since it only inhibits contractions induced by electrical field stimulation at supratherapeutic concentrations.

4.1. Nitrergic innervation of penile small arteries

NO is considered the main inhibitory neurotransmitter in cavernous erectile tissue (Ignarro et al., 1990; Andersson and Wagner, 1995; Burnett, 1997) and large penile arteries supplying glans penis (Liu et al., 1991). In the present study, the NADPH-d positive staining was found both in endothelial cells and adventitia of the penile arteries. In these arteries NO has only a limited role in agonist-induced endothelium-dependent relaxations, while it appears to mediate the neurogenic relaxations in both horse and human small penile arteries (Simonsen et al., 1995, 1997a; Prieto et al., 1998). The staining for nerves immunoreactive for the neural form or NOS I indicates the presence of a dense nitrergic innervation of the penile arterial circulation. Moreover, in the present study the inhibitor of NOS, L-NOARG, inhibited the neurogenic relaxations induced by electrical field stimulation also in endothelium-denuded segments of penile small arteries. These findings extend our previous studies and indicate the presence of a dense nitrergic innervation of the penile arterial circulation supplying corpus cavernosum.

In the present study like in earlier studies of horse and human penile small arteries (Simonsen et al., 1995, 1997a), the NANC relaxations induced by low frequency electrical field stimulation were abolished by L-NOARG, while those induced by high-frequency stimulation were more resistant to the NOS blockade, but abolished by tetrodotoxin. It is unlikely that the inability of L-NOARG to block the neurogenic relaxations induced by high-frequency electrical field stimulation can be ascribed to an insufficient concentration of L-NOARG, since the same concentration of L-NOARG did abolish the non-adrenergic non-cholinergic relaxations of the corpus cavernosum smooth muscle in the present study. Therefore, our findings suggest that NO or a NO-like compound probably mediates the relaxations to low frequency stimulation, while both NO and a still unknown inhibitory NANC transmitter mediate the relaxations of penile small arteries at higher frequencies of electrical field stimulation.

The classical signal transduction pathway ascribed to NO is the activation of soluble guanylyl cyclase and a subsequent increase of the cyclic GMP levels in the smooth muscle cells (Gruetter et al., 1981). Moreover, cyclic GMP appears in erectile tissue to activate protein kinase G type I, since corpus cavernosum strips from cyclic GMP-dependent kinase I deficient infertile mices do not relax to electrical field stimulation (Hedlund et al., 2000). ODQ is considered a specific inhibitor of guanylyl cyclase (Garthwaite et al., 1995), although it also inhibits other heme enzymes such as the NO synthase in concentrations above 10 μM (Feelisch et al., 1999). In the present study, a low concentration (3 μM) of ODQ inhibited the relaxations induced by electrical field stimulation and abolished those induced by the NO donor, *S*-nitroso-*N*-acetylpencillamine, suggesting that NO released by electrical stimulation of dilator nerves causes arterial relaxation largely via a cyclic GMP-dependent mechanism.

4.2. Effect of sildenafil on inhibitory neurotransmission in penile small arteries

Sildenafil has earlier been reported to increase the magnitude of the relaxations of corpus cavernosum induced by electrical field stimulation in human corpus cavernosum tissue, an effect ascribed to an elevation of the cyclic GMP levels during nerve stimulation (Ballard et al., 1998). However, in the present study, sildenafil had no or only modest effects on the magnitude of the relaxation responses induced by electrical field stimulation of penile small arteries. For concentrations of sildenafil (1–10 nM), which is in the low therapeutic concentrations of sildenafil for erectile dysfunction (10–30 nM, Boolell et al., 1996; Langtry and Markham, 1999), the 5-HT preconstrictions in the presence of sildenafil were adjusted to contraction levels similar to those in the vehicle control arteries. However, in the present study, high concentrations (>10 nM) of sildenafil abolished the 5-HT-induced precontraction and therefore, excluded an examination of the effect on the neurogenic relaxations of penile small arteries. Although it is a modested effect, our findings that sildenafil increases the magnitude of the neurogenic relaxations in phenylephrine-contracted corpus cavernosum tissue in contrast to 5-HT-contracted tissue, suggest that this effect of sildenafil in vitro is also dependent on the preconstrictor applied.

Sildenafil enhanced consistently the duration of non-adrenergic non-cholinergic relaxations induced by electrical field stimulation in penile small arteries and corpus cavernosum strips independent of the preconstrictor applied. These observations agree with earlier studies which have described that sildenafil can prolong the relaxations of human corpus cavernosal tissue to nerve stimulation (Ballard et al., 1998). The prolonging effect of sildenafil on the neurogenic relaxations can probably be ascribed to

the delaying effect of sildenafil on the decline of cyclic GMP to basal levels when nerve stimulation is halted. It is the area under the neurogenic relaxations of the penile small arteries which would be expected to correspond to the blood flow and therefore, an increased duration of the neurogenic relaxations is relatively more important than an increased magnitude. Extrapolated to an in vivo situation, an increased duration would probably correspond to a prolonged vasodilation of the penile arteries and, hence entrance of blood into the cavernous sinusoids.

The present results provide the first evidence for an enhancing effect of sildenafil on the non-adrenergic non-cholinergic inhibitory neurotransmission in penile small arteries. The effect of sildenafil on penile small arteries was most pronounced at the lowest frequencies of electrical field stimulation which induces L-NOARG and ODQ-sensitive relaxations. This suggests that sildenafil enhances mainly the endogenous NO-mediated neurogenic relaxations.

Similar to the effect on the endogenous NO-mediated neurogenic relaxations, sildenafil enhanced the duration of relaxation induced by authentic NO in penile arteries, but it had no effect on the magnitude of the relaxations. These findings agree with studies showing an enhancement of sodium nitroprusside relaxation in rabbit corpus cavernosum (Chuang et al., 1998) and of glyceryl trinitrate in isolated aortic rings (Wallis et al., 1999). These functional results, taken together with earlier studies reporting high levels of phosphodiesterase V activity in erectile tissue (Boolell et al., 1996; Taher et al., 1997; Ballard et al., 1998), and enhanced elevation of cyclic GMP levels by NO donors in the presence of sildenafil in corpus cavernosum (Jeremy et al., 1997; Chuang et al., 1998; Park et al., 1998) and arterial tissue (Wallis et al., 1999) from several species, support that the synergistic interaction can be ascribed to inhibition of the breakdown by sildenafil of the cyclic GMP levels increased by NO.

The finding of a synergistic interaction between sildenafil and VIP has not previously been reported in erectile tissue. VIP has been shown to cause relaxation in the rat corpus cavernosum tissue by increasing the cyclic AMP content (Miller et al., 1995). Sildenafil was reported not to change the cyclic AMP content in rabbit corpus cavernosum (Jeremy et al., 1997), but recently it was found to increase cyclic AMP levels in human corpus cavernosum even in the absence of an agonist elevating cyclic AMP (Stief et al., 2000). Phosphodiesterase III is present in erectile tissue (Boolell et al., 1996; Taher et al., 1997), and an increase in cyclic GMP by inhibition of phosphodiesterase V exerted by sildenafil may result in an increased elevation of cellular cyclic AMP stimulated by VIP. Such a mechanism has already been described in rat aortic smooth muscle (Maurice and Haslam, 1990), but Hempelmann et al. (1995) found that combined administration of the NO donor 3-morpholino-sydnonimine (SIN-1) and VIP caused non-synergistic relaxant effect in human penile arteries and corpus cavernosum erectile tissue providing evidence against such a mechanism in erectile tissue. Moreover, sildenafil did not enhance VIP-induced relaxations in endothelium-denuded penile arterial segments in the present study suggesting that the interaction of sildenafil and VIP is endothelium-dependent. The lack of effect of sildenafil on VIP relaxation in endothelium-denuded arteries also suggests that sildenafil has selectivity for enhancement of relaxations elicited by either endogenously released or exogenously added NO.

An alternative explanation for the synergistic interaction of VIP and sildenafil could be that VIP is causing cyclic GMP-mediated relaxations in endothelium-intact preparations. In the present study, both the NOS inhibitor, L-NOARG, and the inhibitor of guanylyl cyclase, ODQ, caused partial inhibition of the VIP-induced relaxations in penile small arteries. These observations suggest that sildenafil increases the magnitude of the endothelium-derived NO-mediated part of the VIP relaxation.

4.3. Vasorelaxant effect of sildenafil in penile small arteries

In the present study, sildenafil was found to induce more potent relaxations ($EC_{50} = 1–10$ nM) compared to what has earlier been reported for human and rabbit cavernous tissue ($EC_{50} = 1–10$ μM, Stief et al., 1998). It could be ascribed to the application of different preconstrictors, since 5-HT was applied in the present studies of the bovine penile arteries, while noradrenaline was used for the evaluation in rabbit and human corpus cavernosum tissue (Stief et al., 1998). However, in bovine corpus cavernosum tissue contracted with 5-HT, sildenafil also induced relaxations with less potency and maximal relaxation compared to the penile small arteries. Although it cannot be excluded that a difference in the concentration applied of 5-HT plays a role, the present results suggest that sildenafil has a more pronounced direct vasorelaxant effect in penile small arteries compared to corpus cavernosum tissue.

Cyclic GMP is hydrolyzed by specific cyclic nucleotide phosphodiesterase-enzymes (Polson and Strada, 1996). Sildenafil, which is a selective inhibitor of phosphodiesterase V, caused potent relaxations in 5-HT-contracted penile small arteries and corpus cavernosum suggesting that it potentiates the effect of basal levels of cyclic GMP. In corpus cavernosum, sildenafil in the absence of exogenous NO was found to increase the cyclic GMP content (Jeremy et al., 1997; Park et al., 1998), although in human corpus cavernosum tissue at resting tension sildenafil increased also the cyclic AMP content (Stief et al., 2000). That sildenafil is causing relaxation by increasing the content and action of cyclic GMP is supported by our finding that sildenafil-induced relaxations of both penile small arteries and corpus cavernosum tissue were abol-

ished in the presence of the inhibitor of guanylyl cyclase, ODQ.

Apart from being guanylyl cyclase-dependent, the sildenafil relaxation was endothelium-dependent in penile small arteries. These findings are in agreement with studies of other vascular beds where the relaxations induced by phosphodiesterase V inhibitors such as zaprinast and E4021 were demonstrated to be inhibited by endothelial cell removal (Komas et al., 1991; Saeki et al., 1995; Jeffery and Wanstall, 1998). In these latter studies, the relaxations induced by the phosphodiesterase V inhibitors were also reduced by NOS inhibition, suggesting that they produced part of their vasorelaxant effect by inhibiting the breakdown of cyclic GMP produced specifically by the action of endothelium-derived NO (Komas et al., 1991; Saeki et al., 1995; Jeffery and Wanstall, 1998). In contrast, in the present study, even high concentrations of L-NOARG did not influence the concentration–response curves for sildenafil in the penile small arteries. Both the cyclic GMP-pathway and phosphodiesterase V enzyme are present in vascular endothelial cells (Kishi et al., 1994; Saijonmaa and Fyhrquist, 1998). Therefore, our findings may suggest that inhibition of phosphodiesterase V in endothelial cells either promotes the release of a endothelium-derived relaxing factor, probably distinct from NO, and which is involved in the sildenafil-induced relaxations or increases the action of endothelium-derived factors in the smooth muscle cells.

In penile small arteries NO and a non-prostanoid non-NO factor mediate the endothelium-dependent relaxations (Simonsen et al., 1997a; Prieto et al., 1998). In the present study, the experiments with indomethacin and L-NOARG suggest that the acetylcholine relaxation is mediated by both prostanoids, NO, and a non-prostanoid non-NO factor, while prostanoids and to a lesser degree a non-prostanoid non-NO factor are involved in the sildenafil relaxation of bovine penile arteries. These findings and together with the effect of ODQ may suggest that sildenafil increases the endothelial cell release of prostanoids and a non-prostanoid non-NO factor leading to the relaxation of the underlying smooth muscle layer.

In contrast to the erections induced by intracavernous injection of drugs like papaverine and prostaglandin E_1, sildenafil does not lead to erection in the absence of any sexual stimulus (Carter et al., 1998; Langtry and Markham, 1999). Therefore, in connection with treatment of erectile dysfunction, a direct vasorelaxant effect of sildenafil on penile arteries is considered less important compared to the effect on neurotransmission.

4.4. Effect of sildenafil on neurogenic contractions in penile small arteries

Sympathetic adrenergic tone probably maintains penis in a flaccid state, as indicated by the erection induced after injection of α-adrenoceptor blockers (Brindley, 1986; Giuluiano et al., 1993). In the present investigation of bovine penile small arteries like in earlier studies of horse penile small arteries, the inhibition by prazosin of the neurogenic contractions suggest that they are mediated through activation of postjunctional α_1-adrenoceptors (Simonsen et al., 1997b). However, prazosin had less effect on the contractions induced by electrical field stimulation compared to those induced by exogenously added noradrenaline. These findings suggest that further studies should address whether additional neurotransmitters such as adenosine 5′-triphosphate (ATP) and neuropeptide Y contribute to the neurogenic contractions of penile small arteries.

In contrast to prazosin, sildenafil had no effect on the noradrenaline-induced contractions, and it did only inhibit the neurogenic contractions of penile small arteries in concentrations (30 nM–10 μM) in the high end of the therapeutic level to supratherapeutic concentrations. NOS was blocked to inhibit the concomitant release of NO from vasodilator nerves by electrical field stimulation, resulting in in vitro conditions attempted to be similar to the in vivo situation where sympathetic tone keeps penis in the flaccid state. In vivo studies suggest that sildenafil will not lead to erections in the absence of sexual stimulus (Carter et al., 1998), and therefore the modest effect on the neurogenic contractions of penile small arteries by concentrations corresponding to therapeutic levels of sildenafil, supports that sildenafil selectively enhances neurogenic relaxations.

In summary, the functional and immunocytochemical results of the present study give direct evidence for a nitrergic innervation of bovine small penile arteries. NO-mediated and guanylyl cyclase-dependent relaxations of penile small arteries, considered to be associated with the vasodilatation leading to erection, are selectively increased by the inhibitor of phosphodiesterase V, while sildenafil has no direct effect on the neurogenic contractions.

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