Comparative effects of dilator drugs on human penile dorsal artery and deep dorsal vein

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ABSTRACT
The present study was designed to characterize the response of human penile dorsal artery and deep dorsal vein to dilator drugs used in the diagnosis and treatment of erectile dysfunction with special emphasis on the effects on sympathetic neurotransmission. Ring segments of penile dorsal artery and deep dorsal vein were obtained from 20 multi-organ donors during procurement of organs for transplantation. The rings (3 mm long) were suspended in organ bath chambers for isometric recording of tension. We then studied the relaxant responses to prostaglandin E₁ (PGE₁), vasoactive intestinal peptide (VIP), papaverine (PV), sodium nitroprusside (SNP) and linsidomine chlorhydrate (SIN-1), and analysed the effects of these drugs on contractions induced by stimulation of perivascular sympathetic nerves. In artery and vein rings contracted by noradrenaline, all the drugs tested caused concentration-dependent relaxation. The order of potencies in terms of IC₅₀ values (concentration of agonist causing 50% of the maximal relaxation) was PGE₁ > VIP > SNP > SIN-1 > PV. Both arteries and veins contracted to electrical field stimulation (15 V, 0.5–2 Hz, 0.2 ms duration for 15 s) in a frequency-dependent manner. All relaxant drugs caused concentration-dependent inhibition of neurogenic contractions; the relative order of potencies was PGE₁ > VIP > SNP > SIN-1 = PV. It is concluded that inhibition of sympathetic activity constitutes an effective relaxing mechanism in penile dorsal artery and vein. Modulation of sympathetic activity together with the direct effects on smooth muscle should be considered to evaluate adequately the efficacy of relaxant drugs to increase human penile blood supply.

INTRODUCTION
Intracavernosal injection of dilator drugs is particularly helpful in the diagnosis and treatment of erectile dysfunction [1]. Some of the drugs proposed include prostaglandin E₁ (PGE₁), vasoactive intestinal peptide (VIP), papaverine (PV), sodium nitroprusside (SNP) and linsidomine chlorhydrate (SIN-1). It is assumed that these substances induce a long-lasting increase in arterial inflow to the penis by relaxing smooth muscle through different mechanisms. In addition to the direct effects on smooth muscle, other mechanisms such as inhibition of sympathetic vasoconstriction are likely to be involved in relaxation. For instance, SIN-1, a nitric oxide donor, induces relaxation of human corpus cavernosum and inhibits neurogenic evoked contractions [2]. Because stimulation of the lumbar sympathetic chain produces detumescence in various animal species [3–5], the flaccid

Key words: constriction, penile vessels, relaxation, sympathetic neurotransmission.
Abbreviations: PGE₁, prostaglandin E₁; PV, papaverine; SIN-1, linsidomine chlorhydrate; SNP, sodium nitroprusside; VIP, vasoactive intestinal peptide.
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The state of the penis has been considered to depend on activation of adrenergic nerves through the release of noradrenaline acting on $\alpha_1$-adrenoceptors [6]. Therefore, information concerning the combined effects on smooth muscle and inhibition of sympathetic activity may represent an effective approach to evaluate the efficacy of these drugs to increase penile blood supply. The fact that the venous drainage from the two corpora cavernosa is primarily through the deep dorsal vein [7] raises the possibility that the mechanisms responsible for relaxation of smooth muscle of this vein could be similar to those in penile arteries to ensure a continuous exchange of blood in the penis even during full erection. We have recently provided functional evidence that the human penile deep dorsal vein is an active component of the penile vascular resistance [8]. Thus it seemed important to us to determine not only the responses of arteries but also those of veins to different dilator substances with special emphasis on neurogenic mediated responses. Such an assessment could be relevant in the understanding of the effects of these drugs on the diagnosis and treatment of erectile dysfunction. We designed this study to characterize the effects of PGE$_1$, VIP and PV on human dorsal artery and deep dorsal vein. The results were compared to those obtained in the same vessels with the nitric oxide donors SIN-1 and SNP.

**METHODS**

Penile dorsal arteries and deep dorsal veins were obtained from 20 multi-organ donors during procurement of organs for transplantation (age range 17–71 years). The study was approved by the ethics committee of our institution. The vessels were immediately placed in chilled Krebs–Henseleit solution, and rings 3 mm long were cut under a dissecting microscope (Heerbrugg, Switzerland). Two stainless steel L-shaped pins, 100 $\mu$m in diameter, were introduced through the lumen of the ring. One pin was fixed to the wall of the organ bath, while the other was connected to a force-displacement transducer (Grass FT03). Changes in isometric force were recorded on a Macintosh computer by use of Chart v 3.4/s software and MacLab/8e data acquisition system (AD Instruments). Each ring was set up in a 4 ml bath containing modified Krebs–Henseleit solution of the following composition: NaCl, 115 mM; KCl, 4.6 mM; MgCl$_2$, 2.5 mM; CaCl$_2$, 2.5 mM; NaHCO$_3$, 25 mM; glucose, 11.1 mM and disodium–EDTA, 0.01 mM. The solution was equilibrated with 95% O$_2$ and 5% CO$_2$ to give a pH of 7.3–7.4. Temperature was held at 37 °C. To establish the resting tension for maximal force development, a series of preliminary experiments were performed on rings of similar length and outer diameter which were exposed repeatedly to 100 mmol/l KCl. Basal tension was increased gradually until contractions were maximal. The optimal resting tension was 3.5 g for the artery and 3 g for the vein. The rings were allowed to attain a steady level of tension during a 2–3 h accommodation period before testing. Functional integrity of the endothelium was confirmed routinely by the presence of relaxation induced by acetylcholine (10$^{-7}$–10$^{-6}$ mol/l) or substance P (10$^{-9}$–10$^{-8}$ mol/l) during contraction obtained with noradrenaline (10$^{-7}$–3 × 10$^{-7}$ mol/l).

Electrical field stimulation was provided by a Grass S88 stimulator (Grass Instruments, Quincy, U.S.A.) via two platinum electrodes positioned on each side and parallel to the axis of the vessel ring. To assess the nature of the contractile responses and avoid direct stimulation of smooth muscle, frequency–response relationships were determined on a group of vessels in the presence and absence of 10$^{-4}$ mol/l tetrodotoxin, following procedures described previously [8]. In summary, the protocol was designed to find the optimal stimulation parameters causing a contractile response that was completely eliminated by 10$^{-6}$ mol/l tetrodotoxin. Stimulation was conducted at 15 V for 15 s at frequencies of 0.5, 1 and 2 Hz. A pulse width of 0.2 ms was used. A period of 10–15 min was allowed between stimulations.

To study relaxation, rings were contracted with 10$^{-7}$ to 10$^{-4}$ mol/l noradrenaline. After a stable contraction was obtained, concentration–response curves were determined for PGE$_1$ (10$^{-11}$–3 × 10$^{-7}$ mol/l), VIP (10$^{-11}$–3 × 10$^{-7}$ mol/l), PV (10$^{-9}$–3 × 10$^{-5}$ mol/l), SNP (10$^{-9}$–3 × 10$^{-5}$ mol/l)) and SIN-1 (3 × 10$^{-9}$–3 × 10$^{-4}$ mol/l).

To study the effects of the relaxant drugs on electrical stimulation-induced responses, frequency–response relationships were determined in a separate group of experiments. After an initial stimulation at 1 Hz the vessel rings were consecutively incubated with increasing concentrations of the tested relaxant drugs for 10 min before another stimulation was given. As a control, four consecutive stimulations were given to a group of untreated rings at identical intervals. Less than 10% variability in magnitude of electrical field stimulation-induced contractions was observed for a given ring during four consecutive sets of control stimulations.

**Drugs**

The following drugs were used: tetrodotoxin, prazosin hydrochloride, noradrenaline hydrochloride, acetylcholine chloride, prostaglandin E$_1$ (PGE$_1$), papaverine hydrochloride (PV), vasoactive intestinal peptide (VIP), sodium nitroprusside (SNP) (Sigma Chemical Co., St. Louis, MO, U.S.A.) and lisinidomine chlorhydrate (SIN-1) (ICN Pharmaceuticals Inc., Costa Mesa, CA, U.S.A.). All drugs were dissolved in Krebs solution. Drugs were added to the organ bath in volumes of less than 70 $\mu$l.
Stock solutions of the drugs were freshly prepared every day, and kept on ice throughout the experiment.

Data analysis
Contractions are reported in absolute tension (g) or as a percentage of response to KCl (100 mmol/l). Relaxation is expressed as a percentage of the noradrenaline-induced contraction. IC_{50} values (concentration of agonist at which half-maximum relaxation occurs) were determined from individual concentration–response curves by non-linear regression analysis. Concentration–response curves of the tested agonists or frequency–response relationships were performed in rings obtained from the same patient; the responses obtained in each patient were averaged to yield a single value. Therefore, all n values are presented as the number of individuals from whom the rings were obtained. For electrical stimulation experiments, in which the same rings were stimulated in the absence and presence of antagonists, a paired t-test was used. Statistical significance was accepted at P < 0.05.

RESULTS

Relaxation
PGE_{1}, PV and VIP induced concentration-dependent relaxation in arteries and veins (Figure 1). The relative order of potencies in terms of IC_{50} values was PGE_{1} = VIP > PV for both arteries and veins. The IC_{50} values for these agents, as well as the maximal responses induced, are given in Table 1. Notice that differences in the relative order of potencies, expressed in terms of IC_{50} values, were not significant between arteries and veins. The two nitric oxide donors SNP and SIN-1 also induced concentration-dependent relaxation (Figure 1 and Table 1).

Responses to 100 mmol/l KCl were 2920 ± 160 mg in artery segments (n = 8) and 3780 ± 185 mg in vein segments (n = 8).

Responses to electrical stimulation
Both arteries and veins contracted to electrical field stimulation in a frequency-dependent manner (Figure 2). Because these contractions were abolished by tetrodotoxin (10^{-6} mol/l) and prazosin (10^{-6} mol/l), it is assumed that the effect was due to the release of noradrenaline from adrenergic nerves acting on α_{1}-adrenoceptors.

Figure 3(a) illustrates typical recordings comparing the response of dorsal artery and deep dorsal vein from the same donor patient to electrical field stimulation (1 Hz) and the inhibitory effects of PGE_{1}, VIP and PV on these responses. The relative order of potencies in terms of the lowest concentration inducing significant inhibition was PGE_{1} = VIP > PV. Differences in maximal inhibitions of contractions and in threshold concentrations of the three agents were not significant between arteries and veins (Figure 3b).

The nitric oxide donors SNP and SIN-1 induced significant inhibition of neurogenic responses at 10^{-4} mol/l and 10^{-5} mol/l respectively in arteries and veins (Figure 4). Almost complete inhibition of neurogenic contraction was achieved at concentrations of 10^{-3} mol/l SNP and 10^{-5} mol/l SIN-1.

DISCUSSION

The present study describes the responses of human dorsal artery and deep dorsal vein, two main vessels of the penis, to neurogenic stimulation and to some vasodilators potentially useful in the treatment of erectile dysfunction. We were able to obtain stable results from vessels taken immediately after death, and sympathetic constriction as well as full concentration–response curves to various substances were consistently obtained. The results demonstrate that all substances tested induce relaxation of dorsal artery and deep dorsal vein contracted with noradrenaline, and inhibit contractions induced by electrical field stimulation.

A main finding of our study is the marked contraction of penile arteries and veins in response to electrical field stimulation. This contraction is apparently mediated by
Table 1  Geometric mean IC$_{50}$ values and maximal responses ($E_{\text{max}}$) to dilator substances in penile dorsal artery and deep dorsal vein

<table>
<thead>
<tr>
<th>Substance</th>
<th>Artery</th>
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<th>Vein</th>
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<tbody>
<tr>
<td></td>
<td>IC$_{50}$ (mol/l)</td>
<td>$E_{\text{max}}$ (%)</td>
<td></td>
<td></td>
<td>IC$_{50}$ (mol/l)</td>
<td>$E_{\text{max}}$ (%)</td>
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<tr>
<td>PGE$_1$ (n = 6)</td>
<td>$7.1 \times 10^{-10}$</td>
<td>96 ± 3</td>
<td></td>
<td></td>
<td>$7.2 \times 10^{-10}$</td>
<td>92 ± 2</td>
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<tr>
<td>VIP (n = 6)</td>
<td>$1.6 \times 10^{-9}$</td>
<td>100</td>
<td></td>
<td></td>
<td>$1.2 \times 10^{-9}$</td>
<td>97 ± 1</td>
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<tr>
<td>PV (n = 4)</td>
<td>$6.2 \times 10^{-7}$</td>
<td>100</td>
<td></td>
<td></td>
<td>$5.2 \times 10^{-6}$</td>
<td>100</td>
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<tr>
<td>SNP (n = 5)</td>
<td>$6.8 \times 10^{-7}$</td>
<td>100</td>
<td></td>
<td></td>
<td>$7.3 \times 10^{-6}$</td>
<td>84 ± 4</td>
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<tr>
<td>SIN-1 (n = 6)</td>
<td>$2.8 \times 10^{-7}$</td>
<td>93 ± 4</td>
<td></td>
<td></td>
<td>$1.9 \times 10^{-6}$</td>
<td>82 ± 6</td>
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Figure 2  Contractile effects of electrical field stimulation on penile dorsal artery and deep dorsal vein in the absence (n = 8) and presence of tetrodotoxin ($10^{-6}$ mol/l; n = 6) or prazosin ($10^{-6}$ mol/l; n = 5)

Values are means ± S.E.M. shown by vertical bars.

dr. The release of the adrenergic transmitter, which in turn activates the $\alpha$$_1$-adrenergic receptor. The evidence for this is that tetrodotoxin and prazosin inhibit the response. Unlike resistance vessels, which alter their calibre in response to local regulatory mechanisms, conduit vessels such as those used in this study are mainly regulated through the sympathetic nervous system [9]. Both the dorsal artery and deep dorsal vein show a high responsiveness to adrenergic stimulation and it is suggested that these vessels may have an outstanding contribution to the regulation of penile blood flow when sympathetic activity or plasma catecholamines are increased.

The results show that both PGE$_1$ and VIP relaxed penile vessels and counteracted neurogenic contractions. For both substances, low concentrations ($10^{-9}$–$10^{-8}$ mol/l) were required to reach an inhibition of 50%. The powerful effects of PGE$_1$ and VIP are probably the result of two mechanisms working together. On one hand PGE$_1$ and VIP activate adenylate cyclase leading to an increase in $3',5'$-cAMP and smooth muscle relaxation [10,11]. On the other hand both PGE$_1$ and VIP modulate presynaptic receptors of adrenergic nerves and subsequently inhibit the release of noradrenaline [12,13]. Therefore, it appears that PGE$_1$ and VIP cause relaxation of arteries and veins by direct effects on smooth muscle and by modulation of the sympathetic activity. These combined effects may explain the high efficacy of PGE$_1$ in the treatment of impotence.

Previous studies have shown that VIP relaxes isolated cavernous preparations [14,15] and induces erectile responses in anesthetized dogs [16]. The potent effects of VIP observed in our experiments do not parallel previous clinical findings showing that this peptide failed to induce erectile responses in impotent men [17] and in healthy volunteers [18] but gave good results when combined with the $\alpha$$_1$-adrenoceptor blocking agent phentolamine [19]. The reason for this may be that VIP is more effective in relaxing penile tissues when adrenergic activity is inhibited. Even if this is the case, the relaxation observed in our in vitro experiments in large vessels may not reflect the events occurring further down the vascular tree, in the small vessels of the corpus cavernosum.

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Relaxation of penile arteries and veins

Figure 3 Effects of relaxant drugs on electrical field stimulation-induced contractions
(a) Tracings of contractile responses to electrical field stimulation (1 Hz) of penile dorsal artery and deep vein under control conditions and after incubation with various concentrations of prostaglandin E1 (PGE1), vasoactive intestinal peptide (VIP), and papaverine (PV). (b) Average contractions to electrical field stimulation in the absence and presence of the relaxant drugs.

Figure 4 Attenuation of neurogenic responses induced by nitric oxide donors
(a) Tracings of contractile responses to electrical field stimulation (1 Hz) of penile dorsal artery and deep dorsal vein under control conditions and after incubation with various concentrations of sodium nitroprusside (SNP) and linsidomine chlorhydrate (SIN-1). (b) Average contractions to electrical field stimulation in the absence and presence of SNP and SIN-1.

PV was the first drug used for intracavernosal injection in the treatment of erectile dysfunction [20]. PV is a non-selective inhibitor of phosphodiesterases which induces smooth muscle relaxation by increasing intracellular concentrations of 3',5'-cAMP and 3',5'-cGMP [21]. It has been demonstrated that PV induces calcium depletion of the cytoplasm which results in a relaxation of the smooth muscle cell [22]. The reduction of intracellular calcium concentration induced by PV may be responsible for the decrease in contraction to sympathetic stimulation.
[23]. In agreement with the present results in penile vessels, previous experiments in dogs and monkeys have shown that sympathetic trunk stimulation antagonizes the relaxing action of PV on the cavernous smooth muscle [3]. In our study the effects of PV were significantly less than those of PGE1 and VIP. Extensive clinical trials also indicate that PGE1 is markedly superior to PV with respect to efficacy in the treatment of erectile dysfunction [24].

Nitric oxide (or a substance containing nitric oxide) accounts for the powerful vasodilator effects of endothelium-derived relaxing factor [25,26] and it may be of pathophysiological significance in several disease states [27]. Nitric oxide induces vasorelaxation by activating soluble guanylate cyclase and, as a result, increasing the level of cGMP within vascular smooth muscle [28]. Compounds that stimulate the soluble form of the enzyme guanylate cyclase counteract neurogenic contractions, probably due to inhibition of neurotransmitter to vascular smooth muscle at the prejunctional level [29]. Localization of nitric oxide synthase in penile arteries [30,31] and veins [32] implies that nitric oxide may be the major mediator of erection [33,34]. In addition, nitric oxide from both neural and endothelial sources modulates neurogenic responses of human penile deep dorsal vein [8]. Thus, nitric oxide donors such as SIN-1 and SNP have been proposed to treat impotence [35,36]. Indeed, our experiments show that both nitric oxide donors induce dilation in dorsal arteries and veins and inhibit neurogenic contractions, SNP being the most potent. However, the effects of SNP and SIN-1 were smaller than those observed with PGE1, thus supporting clinical findings indicating that both SIN-1 and SNP show a markedly lower response, compared with PGE1, in the treatment of erectile failure [24,36].

A novel functional consequence of this study is that the responsiveness of the deep dorsal vein is quite similar to that observed in the dorsal artery. It is generally accepted that during erection there is relaxation of arteries, increased intracorporal pressure and reduction of venous outflow mainly due to compression of venular plexus by engorgement of cavernous spaces [37]. However, large veins such as the deep and superficial dorsal veins, located outside the thick envelope that forms the tunica albuginea, are protected from compression during erection. Thus, from a theoretical point of view, penile large veins must be able to accommodate the increase in blood flow during the administration of dilator drugs inducing erection, and to reduce their calibre when sympathetic activity and detumescence are present. This proposal would explain the equivalent responsiveness of penile large arteries and veins observed in the present experiments.

In conclusion, the present study has shown that the substances tested cause different degrees of relaxation of penile dorsal artery and deep dorsal vein by direct effects on smooth muscle and by modulation of sympathetic activity. Ranked by 50% effective concentration, PGE1 and VIP were more potent than SNP, which in turn was more potent than SIN-1 and PV. These differences in the in vitro ability of vasodilators to prevent sympathetic constriction or to relax precontracted vessels may have therapeutic implications. A high sympathetic tone in some patients with impotence may explain the large variability of the individual responses to intracavernosal injections of relaxant drugs. Although the results cannot predict the overall effect of vasodilators in disease states, our study may represent a valid approach to evaluate the effects of vasoactive drugs in the human penile circulation.

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