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Mechanisms of the relaxant effect of vardenafil in rat penile arteries

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ABSTRACT

The aim of the present study was to investigate the mechanisms underlying the vasorelaxation induced by the selective phosphodiesterase 5 (PDE5) inhibitor vardenafil in rat penile small arteries. Segments of the rat dorsal penile artery were mounted in microvascular myographs for isometric tension recording. Concentration–response curves for vardenafil (1 nM–3 μM) and other PDE inhibitors (sildenafil, rolipram and milrinone) were constructed by adding cummulative concentrations of the drugs to arteries precontracted with phenylephrine. The effect of mechanical endothelial cell removal and of selective blockers of the nitric oxide (NO)/cGMP pathway and K^+ channels were evaluated on the vardenafil relaxant responses. Vardenafil was the most potent of the four PDE inhibitors tested that maximally relaxed penile arteries, pD_2 and maximum relaxation being 6.96±0.08 and 97±1% (n=48), respectively. Blockade of guanylate cyclase with ODQ (5 μM), mechanical removal of the endothelium or inhibition of NO synthase with L-NOARG (100 μM) markedly reduced vardenafil-induced relaxations, without altering maximum response. Inhibitors of both the cGMP-dependent (PKG) and the cAMP-dependent (PKA) protein kinases, Rp-8-Br-PET-cGMPS (5 μM) and Rp-8-CPT-cAMPS (50 μM), respectively, both reduced vardenafil relaxant responses and the later abolished that of rolipram. Vardenafil-elicited relaxation was reduced by the selective inhibitor of the large-conductance Ca^{2+}-activated K^+ channels (BK_{Ca}), iberiotoxin (30 nM) and also by the ATP-sensitive K^+ channel (K_{ATP}) inhibitor, glibenclamide (1 μM). Vardenafil induces a potent vasodilatation in rat penile arteries that is partially dependent on the endothelium and the NO/cGMP pathway and involves activation of both BK_{Ca} and K_{ATP} channels.

1. Introduction

The nitric oxide (NO)/cGMP signalling pathway plays a critical role in the physiology of penile erection and also in the pharmacological management of erectile dysfunction. Thus, NO produced by constitutive neuronal and endothelial NO synthase (NOS) is released from nerves and endothelium upon sexual stimulation and relaxes corpus cavernosum and arterial smooth muscle to increase blood flow to the cavernous sinusoids and elicit penile erection (Andersson and Wagner, 1995; Prieto, 2008). NO activates smooth muscle soluble guanylate cyclase and the resultant increased intracellular cyclic guanylate monophosphate (cGMP) levels stimulate the cGMP-dependent protein kinase (PKG) that in turn lowers intracellular Ca^{2+} concentration and/or the Ca^{2+} sensitivity of the contractile machinery to produce relaxation (Schlossmann et al., 2003). cGMP is rapidly inactivated to GMP by the activity of cyclic nucleotide phosphodiesterases (PDEs), PDE5 being the main cGMP catalyzing enzyme in penile smooth muscle (Uckert et al., 2006). This enzyme is the most prominent target of the NO/cGMP signalling cascade identified so far for the pharmacological intervention in erectile dysfunction. Thus, selective inhibitors of PDE5 are safe and well tolerated drugs currently used in the oral therapy of erectile dysfunction and more recently in pulmonary artery hypertension (Goldstein et al., 1998; Uckert et al., 2006), and these drugs differ in their selectivity, efficacy, side effects and pharmacokinetics (Uckert et al., 2006).

PDE5 is abundant in the smooth muscle layer of human penile cavernous and helicine arteries (Waldkirch et al., 2005), where the functional effects of the selective inhibitor sildenafil have been investigated in relation to the involvement of the vascular endothelium and the L-arginine/NO pathway (Simonsen et al., 2001; Prieto et al., 2006a,b). Thus, sildenafil is a potent vasodilator of penile arteries that amplifies the relaxing effects of endothelial-derived NO (Prieto et al., 2006b). Ca^{2+}-activated K^+ channels (K_{Ca}) channels, which are downstream mediators of the NO (NO)/cGMP signalling cascade leading to the relaxation of penile erectile tissues (Simonsen et al., 1995; Prieto et al., 1998), are also involved in the vasorelaxant effect of sildenafil (Prieto et al., 2006a).

Whereas the *in vivo* pro-erectile action and *in vitro* effects of vardenafil in corpus cavernosum are well characterized (Sáenz de Tejada et al., 2001; Giuliano et al., 2003; Lau and Adaikan, 2006), studies on the effects of vardenafil in penile arteries are still missing. For this purpose, the present *in vitro* study was designed to investigate

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the mechanisms underlying the relaxation induced by vardenafil in rat penile small arteries.

2. Materials and methods

2.1. Dissection and mounting

The investigation conforms to the National Guide for the Care and the Use of Laboratory Animals. Penises from male Wistar rats (12–16 weeks old) were excised immediately after sacrifice and placed in cold physiological saline solution (PSS) of the following composition (in mM): NaCl 119, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.17, $MgSO_4$ 1.18, $CaCl_2$ 1.5, ethylenediaminetetraacetic acid 0.027 and glucose 11. Throughout the subsequent dissection the penis was bathed with cold PSS (4 °C) gassed with 5% CO_2/95% O_2 to maintain pH at 7.4. Dorsal penile arteries were dissected by carefully removing the connective and fat adhering tissue, as previously described (Villalba et al., 2007), and arterial segments about 2 mm long were mounted as ring preparations on 40 μm wires in double vascular myographs for isometric tension recording (J.P. Trading, Denmark).

Arteries were allowed to equilibrate in PSS at 37 °C for about 30 min. The relationship between resting wall tension and internal circumference L_{100}, corresponding to a transmural pressure of 100 mm Hg for a relaxed vessel *in situ*, was calculated. The arteries were set to an internal circumference L_1, as determined by the equation, $L_1 = 0.9 \times L_{100}$, since force development is close to maximal at this internal circumference.

2.2. Experimental procedure

The contractile ability of the vessels was tested at the beginning of each experiment by stimulating them twice with KPSS, which is equivalent to PSS but has NaCl exchanged with KCl on an equimolar basis, giving a final concentration of 123.7 mM K^+. Concentration–response curves for vardenafil and other PDE inhibitors (sildenafil, rolipram and milrinone) were constructed in arteries precontrated with phenylephrine (1–3 μM). The role of endothelial cells in the vardenafil-induced relaxation was tested in arteries in which the endothelium was mechanically removed, as previously described (Prieto et al., 1998). After a first control concentration–response curve to vardenafil, endothelium was mechanically removed by guiding a human hair inside the vessel lumen and gently moved forth and back several times. The artery was then challenged with KPSS to check its viability and the absence of endothelium was verified by the lack of relaxation to 10 μM acetylcholine. Thereafter a second concentration–response curve for vardenafil was constructed in the same artery. To investigate the involvement of the NO/cGMP signalling pathway the arteries were incubated for 30 min with 5 μM of the guanylate cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), or 100 μM of the NOS inhibitor, N^G-nitro-L-arginine (L-NOARG). The role of PKG and of the cAMP-dependent protein kinase (PKA) on the relaxations elicited by vardenafil was also assessed by incubating the arteries for 45 min with the inhibitor of PKG, β-Phenyl-1, N2-etheno-8-bromoguanosine-3′,5′-cyclic monophosphorothioate (5 μM, Rp-8-Br-PET-cGMPS), or the inhibitor of PKA, 8-(4-Chlorophenylthio) adenosine-3′,5′-cyclic monophosphorothioate, Rp-isomer (50 μM, Rp-8-CPT-cAMPS). The role of K^+ channels on vardenafil relaxant responses was assessed by using the selective blockers of large and small-conductance K_{Ca} channels, iberiotoxin (30 nM) and apamin (3 μM), respectively, and of K_{ATP} channels, glibenclamide (1 μM).

2.3. Drugs

Apamin, glibenclamide, iberiotoxin, milrinone, phenylephrine hydrochloride and N^G-nitro-L-arginine (L-NOARG) were purchased from Sigma (Spain) and β-Phenyl-1, N2-etheno-8-bromoguanosine-3′,5′-cyclic monophosphorothioate, Rp-isomer (Rp-8-Br-PET-cGMPS) and 8-(4-Chlorophenylthio) adenosine-3′,5′-cyclic monophosphorothioate, Rp-isomer (Rp-8-CPT-cAMPS) from Biolog Life Science Institute (Bremen, Germany). 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) and rolipram were purchased from Tocris Cookson (UK). Sildenafil citrate was synthesized by Pfizer (Sandwich, Bristol, Kent, UK). Vardenafil was provided by Bayer (Barcelona, Spain).

2.4. Statistical analysis

Mechanical responses of the arteries were measured as force and are expressed as active wall tension (ΔT), which is the increase in force (ΔF) divided by twice the segment length. Relaxant responses are shown as percent of the precontraction induced by phenylephrine. Sensitivity to the agonists is expressed in terms of $pEC_{50} = -\log(EC_{50})$, EC_{50} being the concentration of the agonist required to give half-maximal relaxation. Results are expressed as the mean ± SEM. Statistical differences between means were analyzed by a Student's *t*-test for paired observations. Means of multiple groups were compared by one-way analysis of variance (ANOVA) followed by a Bonferroni method as an *a posterior* test. Probability levels less than 5% were considered significant.

3. Results

3.1. Vasorelaxant effect of vardenafil in penile small arteries

The inhibitors of PDE5, vardenafil and sildenafil, of PDE4, rolipram, and of PDE3, milrinone, evoked sustained relaxations of endothelium-intact penile arteries in a concentration-dependent manner (Fig. 1, Table 1), vardenafil and rolipram being significantly more potent than the other two inhibitors. Moreover, whereas vardenafil and milrinone maximally relaxed penile arteries, sildenafil and rolipram relaxed by

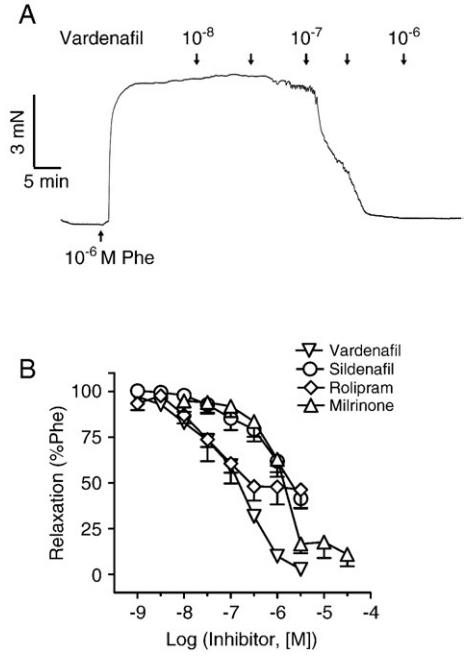

Fig. 1. Relaxant effect of vardenafil and other PDE inhibitors in rat penile arteries. (A) Isometric force recording showing the relaxant effect of vardenafil in a penile small artery of 154 μm. Vertical scale shows force in mN and horizontal bar shows time in min. (B) Average relaxations evoked by the inhibitors of PDE5, vardenafil and sildenafil, of PDE4, rolipram and of PDE3, milrinone. Results (mean ± S.E.M. of 5–48 arteries) are expressed as a percentage of the contraction elicited by phenylephrine.

Table 1
Effect of the PDE5 inhibitors, vardenafil and sildenafil and of the PDE3 and PDE4 inhibitors, milrinone and rolipram, respectively, in rat penile small arteries

	pEC_{50}	Emax (%)	l_1 (μm)	*n*
Vardenafil	6.96±0.08[a,b]	97.2±0.9[1,2]	179±5	48
Sildenafil	6.27±0.23[c]	62.4±4.3[3]	162±5	7
Rolipram	7.33±0.23[d]	53.7±10.2[4]	184±16	9
Milrinone	5.87±0.23	89.07±6.4	175±35	5

Values are mean±S.E.M.; *n* indicates the number of arteries. pEC_{50} is $-\log EC_{50}$, EC_{50} being the concentration of inhibitor required to give half-maximal relaxation. Emax is the maximal relaxation expressed as percentage of the contraction induced by phenylephrine. l_1 is the normalized internal diameter at which experiments were performed. Statistical differences between means were analyzed by one-way analysis of variance (ANOVA) and a Bonferroni method as *a posteriori* test. [a]$P<0.05$ *vs* sildenafil; [b]$P<0.001$ *vs* milrinone; [c]$P<0.01$ *vs* rolipram; [d]$P<0.001$ *vs* milrinone. [1]$P<0.001$ *vs* sildenafil; [2]$P<0.001$ *vs* rolipram; [3]$P<0.05$ *vs* milrinone; [4]$P<0.01$ *vs* milrinone.

Table 2
Effect of endothelium removal and blockers of guanylate cyclase, NO synthase, PKG and PKA on the relaxations elicited by vardenafil in rat penile small arteries

	pEC_{50}	Emax (%)	l_1 (μm)	*n*
Control	6.96±0.22	95.8±2.4	185±14	6
– Endo	6.17±0.14[a]	91.2±2.8	–	6
Control	7.14±0.17	91.9±5.8	198±23	6
5 μM ODQ	6.57±0.13[b]	90.3±5.6	–	6
Control	7.07±0.23	97.8±2.1	205±16	8
100 μM L-NOARG	6.00±0.13[c]	83.2±10	–	8
Control	6.67±0.09	98.6±2.5	164±11	7
5 μM Rp-8-Br-PET-cGMPS	5.82±0.38[a]	88.2±7.1	–	7
Control	7.10±0.44	99.25±2.9	168±11	5
50 μM Rp-8-CPT-cAMPS	5.97±0.30[a]	93.84±1.3	–	5

Values represent mean±S.E.M. of the number *n* of individual arteries. pEC_{50} is $-\log EC_{50}$, EC_{50} being the concentration of vardenafil giving half-maximal relaxation (Emax). Emax is the maximum relaxation expressed as a percentage of the contraction induced phenylephrine. l_1 is the effective lumen diameter of penile resistance arteries at which experiments were performed, determined as $l_1=L_1\pi^{-1}$. Significant differences from controls were analyzed by a paired Student *t*-test. [a]$P<0.05$; [b]$P<0.01$; [c]$P<0.001$.

about 50% the phenylephrine-precontracted arteries at the highest concentrations used (Table 1).

Vardenafil relaxant responses were reproducible in two consecutive concentration–response curves, pEC_{50} values being 6.24±0.17 and 6.01±0.44, and maximal responses 91.0±4.3% and 89.5±3.6% ($n=5$), in a first and a second concentration–response curves, respectively.

3.2. Involvement of the NO/cGMP pathway in vardenafil-induced relaxations

Mechanical removal of endothelium, verified by the absence of relaxant responses to 10 μM acetylcholine (61.5±12.2% and 4.8±2.5, $n=6$, of phenylephrine-induced contraction in endothelium-intact and denuded arteries, respectively), produced a significant inhibition of the vardenafil-induced relaxation, without affecting maximal responses (Fig. 2A, Table 2). Preincubation with the NOS inhibitor L-NOARG (100 μM) and blockade of either guanylate cyclase with ODQ (5 μM) or the inhibitor of PKG, Rp-8-Br-PET-cGMPS (5 μM), significantly reduced the vardenafil relaxant responses without changing maximal relaxations (Fig. 2C, B and D, Table 2).

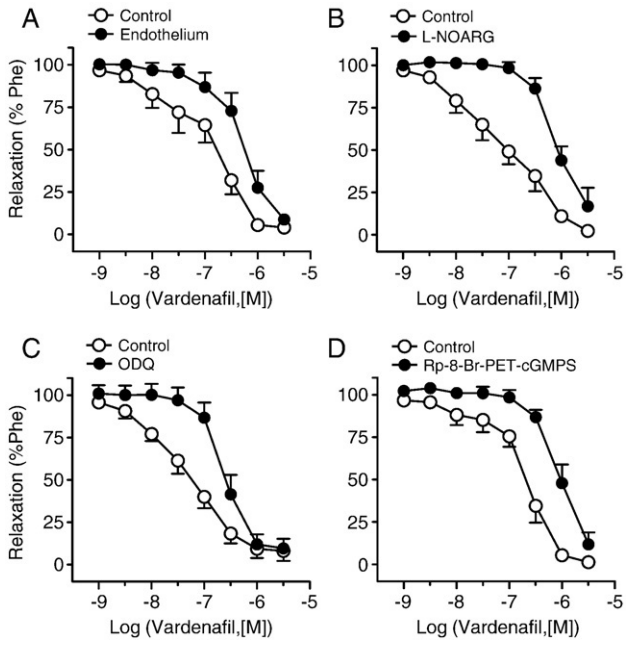

Fig. 2. Effects of (A) endothelial cell removal and inhibition of (B) NOS with L-NOARG (100 μM) (C), guanylate cyclase with ODQ (5 μM) and (D) PKG with Rp-8-Br-PET-cGMPS (50 μM). Results (mean±S.E.M. of 6–8 arteries) are expressed as a percentage of the contraction elicited by phenylephrine.

Moreover, the inhibitor of PKA, Rp-8-CPT-cAMPS (50 μM), abolished rolipram relaxant responses (Fig. 3B) and shifted to the right the concentration–response curves for vardenafil (Fig. 3A, Table 2).

3.3. Effects of K^+ channel blockers

In order to evaluate the role of K^+ channels on vardenafil relaxant responses, the selective blockers of large and small-conductance K_{Ca} channels, iberiotoxin and apamin, respectively, and of K_{ATP} channels, glibenclamide, were examined. Iberiotoxin (30 nM) inhibited the relaxations induced with vardenafil, (Fig. 4A, Table 3), whereas apamin was without effect (Fig. 4B, Table 3). Blockade of K_{ATP} channels with glibenclamide (1 μM) significantly reduced vardenafil-induced relaxations without altering maximum response (Fig. 4C, Table 3).

4. Discussion

By selectively inhibiting the cGMP-specific PDE5, PDE5 inhibitors such as vardenafil and sildenafil, compensate for the reduced NO bioavailability, cGMP production and impaired penile perfusion in erectile dysfunction. The present *in vitro* experiments investigated the vasodilator effect of vardenafil in penile arteries in a comparative analysis with other PDE inhibitors and demonstrate that this agent was the most potent among the 4 inhibitors tested that maximally relaxed penile arterial tissue. In addition, our data confirm the 10 times higher potency of vardenafil compared to sildenafil, reported for the inhibition of cGMP hydrolysis by PDE5 (Bischoff, 2004) and for the relaxation and enhancement of the endothelial and nerve-mediated

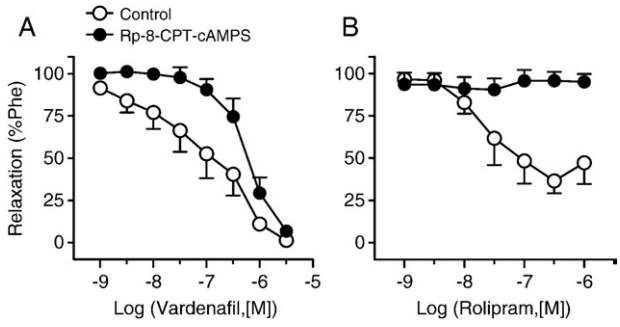

Fig. 3. Effects of the Rp-8-CPT-cAMPS (50 μM), inhibitor of the cAMP-dependent protein kinase A (PKA), on the concentration–relaxation curves to (A) vardenafil and (B) rolipram in rat penile small arteries. Results (mean±S.E.M. of 6–8 arteries) are expressed as a percentage of the contraction elicited by phenylephrine.

Table 3
Effect of K_{Ca} and K_{ATP} channel blockers on the relaxations elicited by vardenafil in rat dorsal penile arteries

	pEC_{50}	Emax (%)	l_1 (μm)	*n*
Control	6.44±0.12	99.5±4.11	168±4	4
30 nM iberiotoxin	5.82±0.19[a]	94.2±0.89	–	4
Control	6.24±0.19	96.4±1.16	174±10	5
3 μM apamin	6.30±0.17	95.8±2.84	–	5
Control	7.16±0.19	99.5±2.04	155±12	7
1 μM glibenclamide	6.18±0.15[b]	92.9±2.64	–	7

Values represent mean±S.E.M. of the number *n* of individual arteries. pEC_{50} is $-\log EC_{50}$, being the concentration of vardenafil giving half-maximal relaxation (Emax). Emax is the maximum relaxation expressed as a percentage of the contraction induced phenylephrine. l_1 is the effective lumen diameter of penile resistance arteries at which experiments were performed, determined as $l_1 = L_1\pi^{-1}$. Significant differences from controls were analyzed by a paired Student *t*-test. [a]$P<0.05$; [b]$P<0.01$.

relaxations in human and rabbit corpus cavernosum *in vitro* (Sáenz de Tejada et al., 2001; Giuliano et al., 2003., Lau and Adaikan, 2006). Moreover, vardenafil had an effective and prolonged pro-erectile action in response to cavernous nerve stimulation in rabbits and rats at lower doses than sildenafil (Sáenz de Tejada et al., 2001; Giuliano et al., 2003). Likewise in the latter studies, the inhibitory potency found for vardenafil in rat penile arteries was slightly lower than that reported for the *in vitro* inhibition of PDE5 enzymatic activity in biochemical assays, which is probably due to the poorer diffusion of the drug within the tissue (Giuliano et al., 2003).

The cGMP-dependent relaxation of the erectile tissue during penile erection is not only dependent on the normal function of the peripheral autonomic nerves but also requires the functional integrity of the vascular endothelium. Thus, neural NO-mediated vasodilatation and increased blood flow to the corpus cavernosum produced by parasympathetic activation in the initial phase of erection leads to shear-stress mediated activation of the endothelial lining and release of NO from the endothelium, thus producing further vasodilatation and sustained erection (Hurt et al., 2002; Prieto, 2008). The present results demonstrate that the relaxations evoked by vardenafil in rat penile arteries involve the vascular endothelium and the NO/cGMP/PKG pathway and suggest that this drug amplifies the relaxant effects of basal NO produced by the endothelium. This is in agreement with that earlier reported for sildenafil in penile resistance arteries, although sildenafil relaxant responses were inhibited to a greater extent than those of vardenafil by endothelial cell removal or treatment with inhibitors of NOS and guanylate cyclase (Simonsen et al., 2001; Prieto et al., 2006a). The lesser involvement of the vascular endothelium and the NO/cGMP cascade in vardenafil-induced relaxation does not seem to be exclusive of penile arteries and has also been observed in rabbit corpus cavernosum (Giuliano et al., 2003; Lau and Adaikan, 2006) and rat aorta (Texeira et al., 2006), thus indicating an additional mechanism of relaxation independent of the NO/cGMP pathway. Inhibition of Ca^{2+} influx has recently been suggested to be involved in the vardenafil relaxant responses of rabbit corpus cavernosum and rat aorta (Lau and Adaikan, 2006; Texeira et al., 2006).

Accumulating experimental evidence supports a cross-talk between the cGMP and cAMP signalling pathways in penile erectile tissues. Thus, sildenafil increases cAMP levels in human corpus cavernosum (Stief et al., 2000) and elicits relaxations in penile resistance arteries that are reduced by selective inhibitors of the cAMP-dependent protein kinase (PKA) (Prieto et al., 2006b), these effects being ascribed to the inhibition of the cAMP-specific cGMP-inhibited PDE3. Furthermore, vardenafil has been shown to inhibit PDE3 activity at the micromolar range (Sáenz de Tejada et al., 2001). In the present study, vardenafil relaxant responses were also significantly reduced by an inhibitor of PKA whose selectivity is indicated by the complete blockade of the relaxations elicited by the inhibitor of the cAMP-specific PDE4 rolipram. This, together with the pronounced relaxant effect of milrinone, a selective inhibitor of PDE3, suggests that vardenafil could elicit relaxation through an increase in endogenous cAMP levels mediated by inhibition of PDE3 in rat penile arteries.

Activation of K^+ channels is a powerful mechanism of vascular smooth muscle hyperpolarization and relaxation, and both cGMP an cAMP can modulate the activity of K^+ channels to elicit vasodilatation (Christ, 2002; Schlossmann et al., 2003; Prieto, 2008). Adrenergic vasoconstriction in rat penile arteries is largely dependent on extracellular Ca^{2+} entry in part through voltage-dependent Ca^{2+} channels (Villalba et al., 2007) and therefore, hyperpolarization would be a feasible mechanism for relaxation/reversion of adrenergic tone in these arteries. K_{Ca} channels are activated by intracellular Ca^{2+} and depolarization and are downstream mediators of the NO/cGMP cascade underlying relaxation of vascular and erectile tissue (Simonsen et al., 1995; Prieto et al., 1998). In the present study, the inhibition elicited by iberiotoxin along with the lack of effect of apamin, indicates that large-conductance K_{Ca} (BK_{Ca}) channels are involved in the vardenafil relaxant responses. These results are in agreement with those reported for sildenafil and NO in penile resistance arteries (Prieto et al., 2006a) and consistent with the concept that regulation of BK_{Ca} by NO and cGMP, usually through PKG, is a common mechanism of vasodilatation in vascular smooth muscle including erectile tissue (Schubert and Nelson, 2001; Prieto et al., 2006a).

Interestingly, the relaxant effect of vardenafil in rat penile arteries was significantly reduced by the K_{ATP} inhibitor, glibenclamide. K_{ATP} channels are usually regulated by cAMP-elevating agents in penile resistance (Ruiz Rubio et al., 2004) and other small arteries (Quayle et al., 1997) and therefore, these channels could be involved in the PKA-mediated part of the relaxation evoked by vardenafil in rat penile arteries. However, cAMP-independent regulation of K_{ATP} channels has also been reported for the receptor-mediated vasodilator effect of prostacyclin and adenosine in arterial smooth muscle (Quayle et al., 1997). In addition, recent studies have demonstrated that vardenafil has a powerful cardioprotective effect against ischemia/reperfusion through opening of mitochrondrial K_{ATP} channels (Salloum et al., 2007). Therefore, further studies are needed to clarify the intracellular

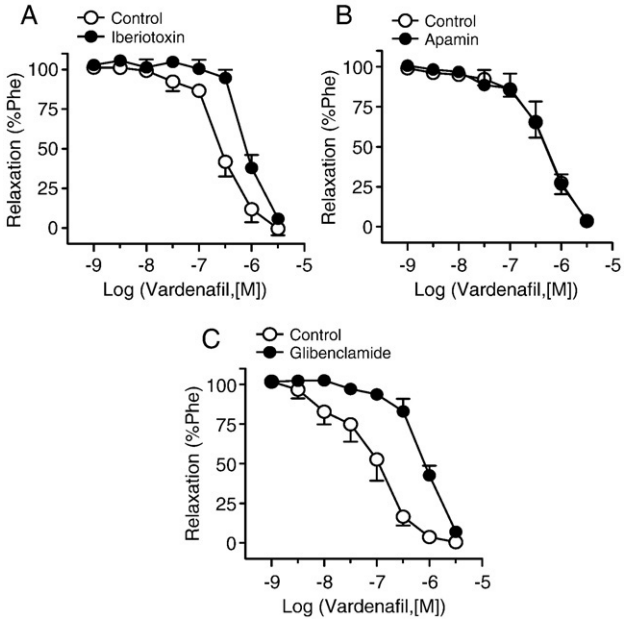

Fig. 4. Effects of the blockers of large and small-conductance K_{Ca} channels (A) iberiotoxin (30 nM) and (B) apamine (3 μM), respectively, and (C) of K_{ATP} channels, glibenclamide (1 μM) on the concentration–relaxation curves to vardenafil in rat penile small arteries. Results (mean±S.E.M. of 4–7 arteries) are expressed as a percentage of the contraction elicited by phenylephrine.

signalling pathways of the vardenafil-induced relaxation leading to activation of K_{ATP} channels in rat penile arteries.

In conclusion, the present study first demonstrates a potent vasodilator effect of vardenafil in rat penile arteries that is partially dependent on the endothelium and the NO/cGMP pathways and involves activation of BK_{Ca} and K_{ATP} channels. The lesser endothelial and NO dependence of the vardenafil-induced vasodilatation may represent a therapeutic advantage in those patients with erectile dysfunction and cardiovascular disease, e.g. diabetic patients, in whom endothelial function and NO bioavailability are impaired.

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References

Andersson, K.E., Wagner, G., 1995. Physiology of penile erection. Physiol. Rev. 75, 191–236.
Bischoff, E., 2004. Vardenafil peclinical trial data: potency, pharmacodynamics, pharmacokinetics, and adverse events. Int. J. Impot. Res. 16, 34–37.
Christ, G., 2002. K^+ channels as molecular targets for the treatment of erectile dysfunction. J. Androl. 23, 10–19.
Giuliano, F., Bernabe, J., Alexandre, L., Niewoehner, U., Haning, H., Bischoff, E., 2003. Pro-erectile effect of vardenafil: in vitro experiments in rabbits and in vivo comparison with sildenafil in rats. Eur. Urol. 44, 731–736.
Goldstein, I., Lue, T.F., Padma-Nathan, H., Rosen, R.C., Steers, W.D., Wicker, P.A., 1998. Oral sildenafil in the treatment of erectile dysfunction. Sildenafil Study Group. N. Engl. J. Med. 338, 1397–1404.
Hurt, K.J., Musicki, B., Palese, M.A., Crone, J.K., Becker, R.E., Moriarity, J.L., Snyder, S.H., Burnett, A.L., 2002. Akt-dependent phosphorylation of endothelial nitric-oxide synthase mediates penile erection. Proc. Natl. Acad. Sci. U. S. A. 99, 4061–4066.
Lau, L.C., Adaikan, G.A., 2006. Mechanisms of direct relaxant effect of sildenafil, tadalafil and vardenafil on corpus cavernosum. Eur. J. Pharmacol. 541, 184–190.
Prieto, D., 2008. Physiological regulation of penile arteries and veins. Int. J. Impot. Res. 20, 17–29.
Prieto, D., Simonsen, U., Hernández, M., García-Sacristán, A., 1998. Contribution of K^+ channels and ouabain-sensitive mechanisms to the endothelium-dependent relaxations of horse penile small arteries. Br. J. Pharmacol. 123, 1609–1620.
Prieto, D., Rivera, L., Benedito, S., Recio, P., Villalba, N., Hernandez, M., Garcia-Sacristan, A., 2006a. Ca^{2+} activated K^+ (KCa) channels are involved in the relaxations elicited by sildenafil in penile resistance arteries. Eur. J. Pharmacol. 531, 232–237.
Prieto, D., Rivera, L., Recio, P., Rubio, J.L., Hernandez, M., Garcia-Sacristan, A., 2006b. Role of nitric oxide in the relaxation elicited by sildenafil in penile resistance arteries. J. Urol. 175, 1164–1170.
Quayle, J.M., Nelson, M.T., Standen, N.B., 1997. ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. Physiol. Rev. 77, 1165–1232.
Ruiz Rubio, J.L., Hernandez, M., Rivera de los Arcos, L., Benedito, S., Recio, P., Garcia, P., Garcia-Sacristan, A., Prieto, D., 2004. Role of ATP-sensitive K+ channels in relaxation of penile resistance arteries. Urology 63, 800–805.
Sáenz de Tejada, Angulo, J., Cuevas, P., Fernández, A., Moncada, I., Allona, A., Lledó, E., Körschen, H.G., Niewöhner, U., Haning, H., Pages, E., Bischoff, E., 2001. The phosphodiesterase inhibitory selectivity and the in vitro and in vivo potency of the new PDE5 inhibitor vardenafil. Int. J. Impot. Res. 13, 282–290.
Salloum, F.N., Takenoshita, Y., Ockaili, R.A., Dauod, V.P., Chou, E., Yoshide, K., Kukreja, R., 2007. Sildenafil and vardenafil but not nitroglicerin limit myocardial infarction through opening of mitocondrial K_{ATP} channels when administrated at reperfusion following ischemia in rabbits. J. Mol. Cell. Cardiol. 42, 453–458.
Schlossmann, J., Fiel, R., Hofmann, F., 2003. Signaling through NO and cGMP-dependent protein kinases. Ann. Med. 35, 21–27.
Schubert, R., Nelson, M.T., 2001. Protein kinases: tuners of the BKCa channel in smooth muscle. Trends Pharmacol. Sci. 22, 505–512.
Simonsen, U., Prieto, D., Sáenz de Tejada, I., Garcia-Sacristan, A., 1995. Involvement of nitric oxide in the non-adrenergic non-cholinergic neurotransmission of horse deep penile arteries: role of charybdotoxin-sensitive K(+)-channels. Br. J. Pharmacol. 116, 2582–2590.
Simonsen, U., Contreras, J., Garcia-Sacristan, A., Martinez, A.C., 2001. Effect of sildenafil on non-adrenergic non-cholinergic neurotransmission in bovine penile small arteries. Eur. J. Pharmacol. 412, 155–169.
Stief, C.G., Uckert, S., Becker, A.J., Harringer, W., Truss, M.C., Forssmann, W.G., Jonas, U., 2000. Effects of sildenafil on cAMP and cGMP levels in isolated human cavernous and cardiac tissue. Urology 55, 146–150.
Texeira, E.C., Priviero, F.B., Clinton Webb, R., 2006. Differential effects of the fosfodiesterase type 5 inhibitors sildenafil, vardenafil, and tadalafil in rat aorta. J. Pharmacol. Exp. Ther. 316, 654–661.
Uckert, S., Hedlung, P., Andersson, K.E., Truss, M.C., Jonas, U., Stief, C.G., 2006. Update on phosphodiesterase (PDE) isoenzymes as pharmacologic targets in urology: present and future. Eur. Urol. 50, 1194–1207.
Villalba, N., Stankevicius, E., García-Sacristán, A., Simonsen, U., Prieto, D., 2007. Contribution of both Ca2+ entry and Ca2+ sensitization to the α1-adrenergic vasoconstriction of rat penile arteries. Am. J. Physiol., Heart. Circ. Physiol. 292, 1157–1169.
Waldkirch, E., Uckert, S., Yildirim, H., Sohn, M., Jonas, U., Stief, C.G., Andersson, K.E., Hedlund, P., 2005. Cyclic AMP-specific and cyclic GMP-specific phosphodiesterase isoenzymes in human cavernous arteries—immunohistochemical distribution and functional significance. World J. Urol. 23, 405–410.