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Mechanisms of direct relaxant effect of sildenafil, tadalafil and vardenafil on corpus cavernosum

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Abstract

Sildenafil, tadalafil, vardenafil and verapamil induced concentration-dependent relaxation of the rabbit corpus cavernosum muscle precontracted with noradrenaline. The maximal relaxation (%) at 20 μM was 61.4±6.9, 32.4±5.4, 100.0±5.5 and 86.6±5.1 (n=5 each) respectively. Pre-incubation of cavernosal muscle strips with N^{ω}-nitro-L-arginine or guanylate cyclase inhibitor, 1H-[1,2,4]oxadiazolo[4,3-a] quinoxalin-1-one (ODQ) but not adenylate cyclase inhibitor, *cis*-*N*-[2-phenylcyclopentyl]-azacyclotridec-1-en-2-amine] (MDL12330A) culminated in only a 20–30% reduction in muscle relaxant action of the 3 phosphodiesterase inhibitors. This suggests that another mechanism of relaxation independent of nitric oxide–cGMP or cAMP pathway was involved. Higher concentrations of sildenafil (100 μM) and vardenafil (10 and 100 μM) produced non-competitive antagonism of noradrenaline-induced contraction characterized by reduced maximal effect. In contrast, tadalafil was devoid of significant effect on noradrenaline. On K^+-depolarized tissues, sildenafil was as potent as vardenafil whereas tadalafil was the least effective in relaxing K^+-induced tone. The maximal relaxation (% of K^+-induced tone) at 20 μM sildenafil, tadalafil and vardenafil was respectively 84.1±6.5, 9.0±19.9, and 88.9±6.2 (n=5 each). In addition, verapamil, sildenafil and vardenafil were more efficacious than tadalafil in reversing tonic contractions by Ca^{2+} channel activator, 1,4,dihydro-2,6-dimethyl-5-nitro-4-[2(triflouromethyl)phenyl]pyridine-3-carboxylic acid methyl ester (BAY K-8644). These results indicate that vardenafil and sildenafil possess direct muscle relaxant potential possibly via inhibiting Ca^{2+} influx through both receptor-operated and voltage-dependent Ca^{2+} channels whereas tadalafil appears capable of inhibiting receptor-operated transmembrane Ca^{2+} entry only.

Keywords: Sildenafil; Tadalafil; Vardenafil; Corpus cavernosum; Signal transduction

1. Introduction

Recent studies have identified at least 11 phosphodiesterase (PDE) families (PDE1-11) with distinct tissue distribution, substrate specificity and drug sensitivity. With respect to substrate specificity, PDE4, 7 and 8 are specific for cAMP whereas PDE5, 6 and 9 are specific for cGMP and the remaining PDEs are of mixed specificity (Francis et al., 2001). In corpus cavernosum tissue, the expression of PDE2, 3, 4, 5 and 11 has been demonstrated and PDE5 is the predominant enzyme (Ballard et al., 1998; Wallis et al., 1999; Fawcett et al., 2000). Inhibitors of PDE5 enzyme such as sildenafil, tadalafil and vardenafil enhance nitric oxide (NO)-induced relaxation of corpus cavernosum smooth muscle and facilitate penile erection by increasing the intracellular cGMP level.

Sildenafil, tadalafil and vardenafil are the three PDE5 inhibitors currently in clinical use for erectile dysfunction. They differ in their selectivity, efficacy, side effects and pharmacokinetics data. Vardenafil is the most potent of the three in inhibiting PDE5 activity while tadalafil was reputed to have extended plasma half-life up to 18 h as compared to 3–4 h in the case of sildenafil and vardenafil (Bischoff, 2004a). In addition to PDE5 inhibition, sildenafil evoked concentration-dependent relaxation in rabbit, human and monkey cavernosal tissues (Lau et al., 2000a; Palea and Barras, 2003). Similar muscle relaxant activity has been observed with vardenafil and tadalafil respectively in

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rabbit and human cavernosum muscle strips precontracted with adrenergic agonists (Giuliano et al., 2003; Uckert et al., 2004). Besides in vitro studies, intracavernous injections of sildenafil (in anaesthetized cat and rabbit) and intravenously administered vardenafil (in anaesthetized rat) have also been shown to cause a dose-related increase in intracavernous pressure and relaxation of penile muscle (Doherty et al., 2001; McAuley et al., 2001; Giuliano et al., 2003).

Furthermore, sildenafil significantly reduced adrenergic transmission in human cavernosal strip suggesting that sildenafil was capable of modulating the cavernosal tone in favour of corpus cavernosum muscle relaxation. The depressant effect on adrenergic transmission was effectively prevented by the addition of N^{ω}-nitro-L-arginine strongly supporting the notion that enhancement of cGMP-mediated nitrergic relaxation can override the excitatory adrenergic transmission (Lau et al., 2000b). However, in monkey corpus cavernosal tissue, sildenafil markedly attenuated the later phase of neurogenic contraction with an action that was characteristic of a calcium antagonist (Lau et al., 2000a). While there is evidence suggesting a mechanism independent of NO–cGMP pathway is involved, the exact mechanism of action underlying the direct relaxant effect of these PDE5 inhibitors has not been fully delineated.

Hence, the present study attempts to characterize the role of NO–cGMP and Ca^{2+} signaling pathways with respect to the pharmacological activity of all 3 compounds that may provide further insights in our understanding of their potentials in pharmacotherapy of erectile dysfunction or other related disease conditions.

2. Materials and methods

2.1. Rabbit corpus cavernosum tissue

Penile erectile tissue was obtained from male New Zealand White rabbits (22–26 weeks old; 3–4 kg body weight). The rabbits were killed by euthanasia with sodium pentobarbital injection (100 mg/kg) through the ear vein in accordance with the guidelines for animal experiments and principles for the care and use of animals in research and teaching established by National University of Singapore.

The entire penis was surgically removed and placed in chilled Tyrode's solution of the following composition (mmol/l): NaCl 137, $NaHCO_3$ 11.9, $CaCl_2$ 1.8, KCl 2.7, $MgSO_4$ 1.1, NaH_2PO_4 0.42 and glucose 5.6.

Corpus cavernosum was carefully dissected, cleared of adherent adipose and muscular tissues and tunica albuginea. The cavernosal strips were mounted under 10 mN resting tension in 25-ml organ baths containing Tyrode's solution bubbled with 95% O_2, 5% CO_2 and maintained at 37 °C. An equilibration period of 1 h was applied for all tissues during which tissues were washed with fresh Tyrode's solution and baseline readjusted to 10 mN.

Tissue responses were measured using isometric transducers (Ugo Basile, Italy) connected to MacLab 4e electronic data acquisition system running chart software version 3.6.4 (ADInstrument, Australia) on a MacIntosh computer.

2.2. Effect of PDE5 inhibitors and verapamil on tone generated by noradrenaline, K^+ or BAY K-8644

Cavernosal tissue was precontracted with 30 μM noradrenaline or 120 mM KCl which produced submaximal contraction. After having obtained a stable plateau of contraction with noradrenaline or KCl, PDE5 inhibitors (sildenafil, tadalafil or vardenafil) were administered in cumulative concentrations. Similarly, in cavernosal strips precontracted with noradrenaline, the concentration response curve to cumulative dosing of verapamil, an L-type Ca^{2+} channel blocker, was obtained. Each cavernosal tissue was used for only one exposure to PDE5 inhibitor since the effect of PDE5 inhibitors was long lasting and persisted for more than two hours despite repeated washings with fresh Tyrode's solution. In parallel experiments, cavernosal tissue strips were incubated for 30 min with 1-H-[1,2,4]-oxadiazolo-[4,3-a]-quinoxalin-1-one (ODQ, a selective inhibitor of soluble guanylate cyclase), N^{ω}-nitro-L-arginine (an inhibitor of NO synthase) or *cis*-*N*-[2-phenylcyclopentyl]-azacyclotridec-1-en-2-amine] (MDL12330A, an inhibitor of adenylate cyclase) before testing PDE inhibitors.

Preliminary investigation reveals that 1,4,dihydro-2,6-dimethyl-5-nitro-4-[2(triflouromethyl)phenyl]pyridine-3-carboxylic acid methyl ester (BAY K-8644), a well characterized L-type Ca^{2+} channel activator, at concentrations ranging from 1 to 100 nM either did not or was weak in eliciting contractile response in cavernosal tissue (data not shown). However, BAY K-8644 (100 nM) evoked contractile tonic responses when the cavernosal preparations were incubated in Tyrode buffer supplemented with 20 mM K^+ (Usowicz et al., 1995). After a sustained response was obtained, cumulative concentration response curves for verapamil and PDE5 inhibitors were determined.

2.3. Effect of PDE5 inhibitors on noradrenaline-induced contraction

To verify if the relaxant effect of PDE5 inhibitor was partly due to α-adrenoceptor antagonism, cavernosal strip was treated with N^{ω}-nitro-L-arginine (50 μM) to block NO synthesis and two consecutive concentration response curves to noradrenaline were constructed in the same preparation without and then with (1 μM, 10 μM or 100 μM) sildenafil, tadalafil or vardenafil. The two concentration response curves were separated by an interval of 90 min. PDE5 inhibitor was incubated for 30 min.

2.4. Drugs and chemicals

All drugs were purchased from Sigma-Aldrich (St Louis, USA) unless otherwise indicated. Sildenafil citrate (Pfizer) and tadalafil (Eli-Lilly) were reconstituted by suspending crushed Viagra (50 mg) and Cialis (20 mg) tablets respectively in water and filtering the biphasic solution. BAY K-8644 was initially prepared as a stock solution in dimethyl sulphoxide (DMSO) and subsequent dilution in deionised water prior to use. The final concentration of DMSO did not exceed 0.1%. DMSO in the concentrations used did not contract or relax the cavernosal muscle. Aqueous stock solutions of noradrenaline, verapamil,

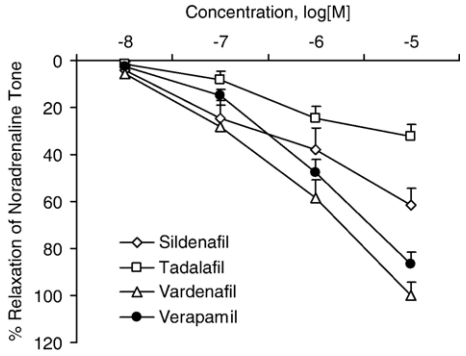

Fig. 1. Sildenafil, tadalafil, vardenafil and verapamil concentration-dependently reversed noradrenaline-induced tone in isolated rabbit corpus cavernosum. Data are the mean±S.E.M.

vardenafil (Bayer), MDL12330A, ODQ and N^{ω}-nitro-L-arginine were prepared and stored at −20 °C. Working concentrations of each compound were prepared freshly on the day of experiment and stored on crushed ice.

2.5. Data analysis

The relaxations are expressed as a percentage decrease in contraction induced by noradrenaline, K^+ or BAY K-8644. Data are expressed as the mean±S.E.M. Statistical comparisons between concentration response curves were made using a one-way analysis of variance (ANOVA), with Bonferroni's correction for multiple comparisons being performed post hoc; a probability level of $P<0.05$ being regarded significant.

3. Results

Sildenafil, tadalafil, vardenafil and verapamil mediated concentration-dependent relaxation in rabbit corpus cavernosum strips challenged with noradrenaline. The estimated maximal relaxation (%) at 20 μM was respectively 61.4±6.9, 32.4±5.4, 100.0±5.5 and 86.6±5.1 ($n=5$; Fig. 1). Pre-treatment with ODQ and N^{ω}-nitro-L-arginine produced rightward shift of the concentration response curve to sildenafil, tadalafil and vardenafil and partial attenuation of the relaxant activity. For instance the relaxant responses to 0.2 μM and 2 μM sildenafil were respectively 6.0±1.8 and 19.8±4.9 in the presence of N^{ω}-nitro-L-arginine and 8.9±2.1 and 18.0±3.3 in the presence of ODQ as compared to 24.6±7.7 and 37.9±9.3 in the absence of either inhibitor. Strikingly similar range of inhibition (by 20–30%) was obtained when sildenafil was substituted with tadalafil and vardenafil (Fig. 2). MDL12330A, an inhibitor of adenylate cyclase, failed to influence the relaxant activity of tadalafil and vardenafil (Fig. 2). Though MDL12330A led to a shift in the concentration response curves of sildenafil to the right, the resultant effect was not statistically significant ($n=4$; $P>0.05$; Fig. 2).

Concentration response curve of noradrenaline in cavernosal strips treated with lower concentration (1 μM) of PDE5 inhibitor was not significantly different from those contractions generated in the absence of PDE5 inhibitor (Figs. 3–5). However, at higher concentrations both sildenafil (Fig. 3) and vardenafil (Fig. 5) produced significant shift of the concentration response curve of noradrenaline to the right with depressed maximal contraction. The maximal contractions produced by noradrenaline were 83.4±9.3 ($n=4$; $P<0.05$) with 100 μM

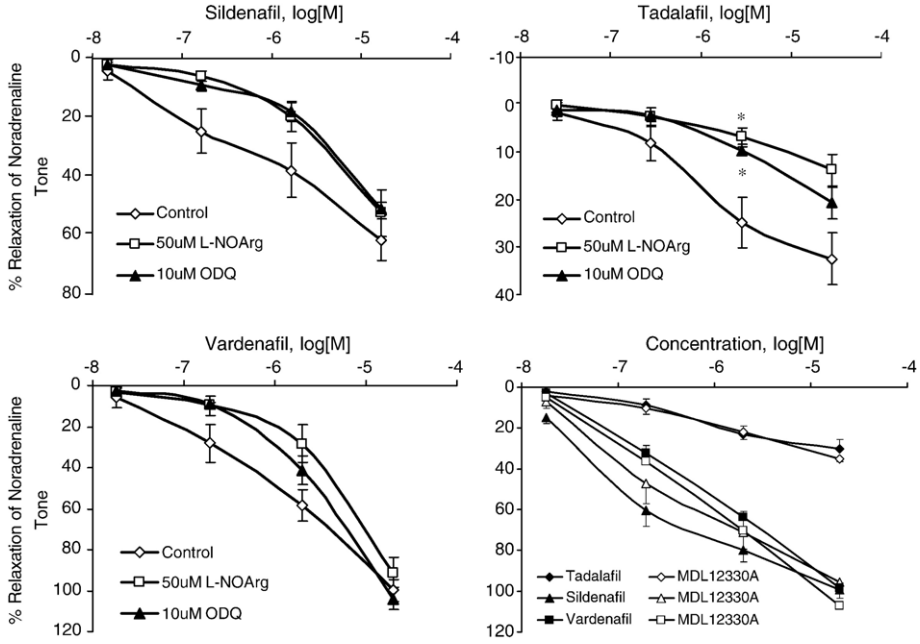

Fig. 2. N^{ω}-nitro-L-arginine (L-NOArg) and ODQ partially inhibited the relaxant effect of sildenafil, tadalafil and vardenafil in rabbit corpus cavernosum smooth muscle whereas MDL12330A was without significant effect. Rabbit corpus cavernosum tissue strips were contracted with 30 μM noradrenaline and exposed to increasing concentrations of sildenafil, tadalafil and vardenafil by cumulative addition. In parallel experiments, tissue strips were first incubated for 30 min with 50 μM L-NOArg or 10 μM ODQ or 10 μM MDL12330A and the relaxation responses to increasing concentrations of sildenafil, tadalafil and vardenafil were determined. Data are the mean±S.E.M. *$P<0.05$ relative to control.

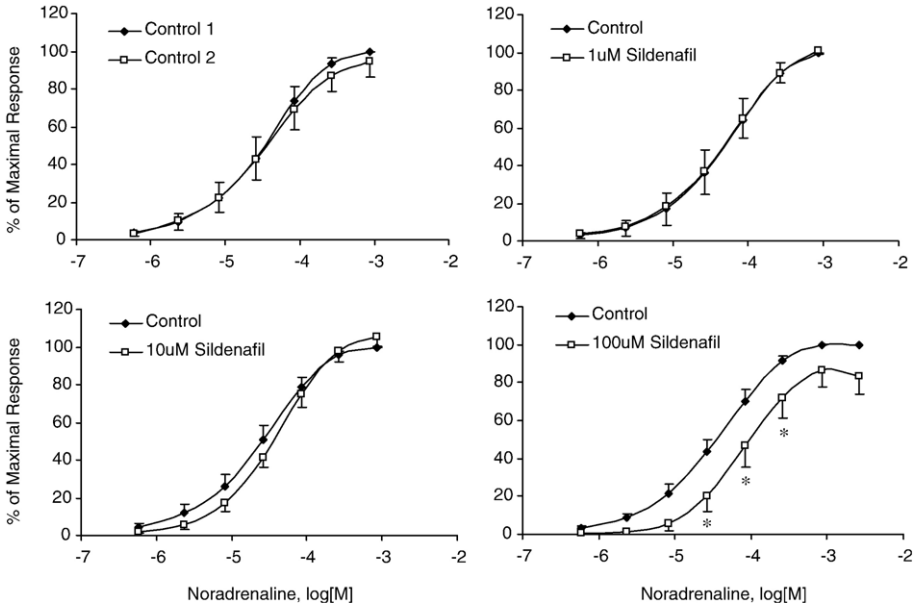

Fig. 3. Reproducibility of two consecutive concentration response curves to noradrenaline in isolated rabbit corpus cavernosum, showing the first (Control 1) and second curve (Control 2) in the presence of N^{ω}-nitro-L-arginine. The two concentration response curves were separated by a 90 min interval, during which tissues were washed every 15 min. In parallel experiments, tissue strips were first treated for 30 min with 1 μM, 10 μM or 100 μM sildenafil and the second concentration response curve to noradrenaline was determined. Data are the mean±S.E.M. *$P<0.05$ relative to control.

sildenafil and significantly lower 73.2±6.1 and 39.7±3.6 ($n=5$ each) with 10 and 100 μM vardenafil respectively. In contrast, as depicted in Fig. 4, tadalafil at 10 μM shifted the concentration response curve of noradrenaline slightly to the left with increased maximal contraction (109.6±3.9; $n=5$; $P>0.05$) which was not statistically significant. At 100 μM tadalafil was devoid of any effect (92.8±7.3; $n=5$; $P>0.05$).

On K^+-depolarized CC strips, both sildenafil and vardenafil were almost equi-potent in inducing relaxation in a concentration-dependent manner while tadalafil managed slight relaxation at the highest concentration tested (Fig. 6). The maximal relaxation (expressed as % of K^+-induced tone) attained by 20 μM sildenafil, tadalafil and vardenafil was respectively 84.1±6.5, 9.0±19.9, and 88.9±6.2 ($n=5$ each).

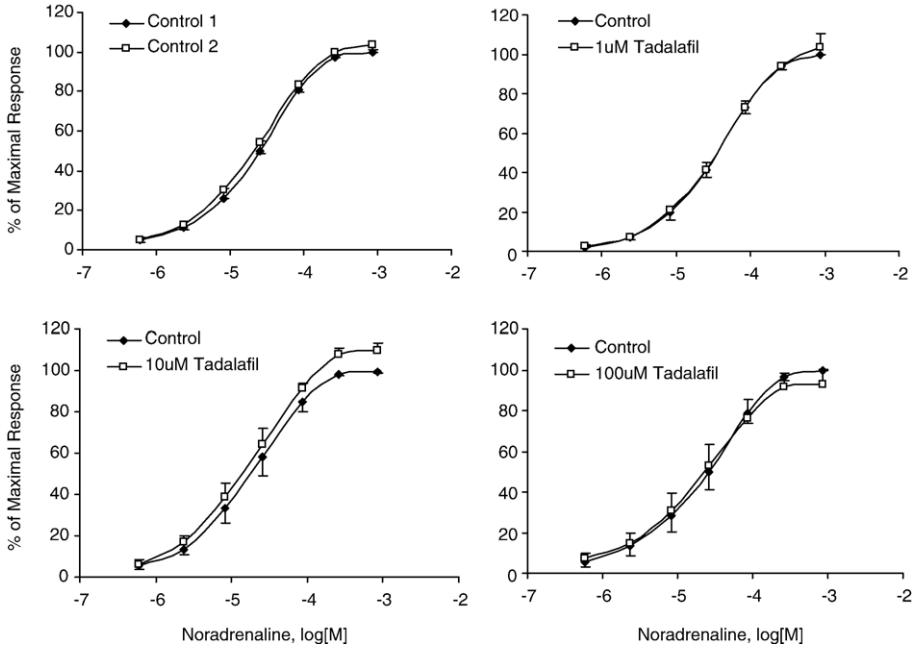

Fig. 4. Reproducibility of two consecutive concentration response curves to noradrenaline in isolated rabbit corpus cavernosum, showing the first (Control 1) and second curve (Control 2) in the presence of N^{ω}-nitro-L-arginine. The two concentration response curves were separated by a 90 min interval, during which tissues were washed every 15 min. In parallel experiments, tissue strips were first treated for 30 min with 1 μM, 10 μM or 100 μM tadalafil and the second concentration response curve to noradrenaline was determined. Data are the mean±S.E.M.

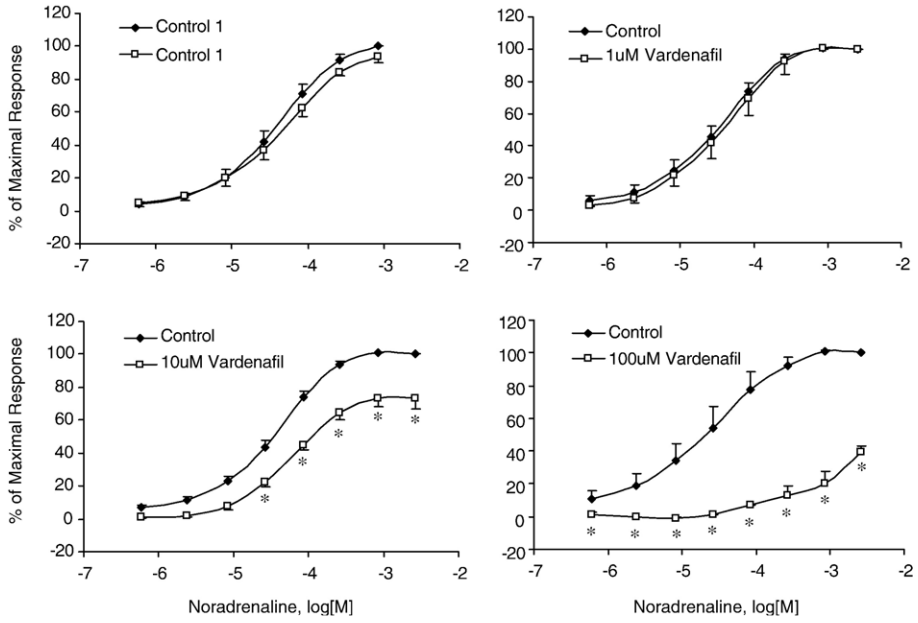

Fig. 5. Reproducibility of two consecutive concentration response curves to noradrenaline in isolated rabbit corpus cavernosum, showing the first (Control 1) and second curve (Control 2) in the presence of N^{ω}-nitro-L-arginine. The two concentration response curves were separated by a 90 min interval, during which tissues were washed every 15 min. In parallel experiments, tissue strips were first treated for 30 min with 1 μM, 10 μM or 100 μM vardenafil and the second concentration response curve to noradrenaline was determined. Data are the mean±S.E.M. *$P<0.05$ relative to control.

The application of 100 nM BAY K-8644 to the cavernosal strips in Tyrode buffer augmented with 20 mM K^+ induced tonic contractions which were effectively suppressed by sildenafil ($n=5$), vardenafil ($n=4$) and verapamil ($n=5$) in a concentration-related manner. Tadalafil at the highest concentration tested partially inhibited the contractile effect of BAY K-8644 (44.6±11.2%; $n=4$; Fig. 7).

4. Discussion

The clinical efficacy from a long series study on sildenafil, tadalafil and vardenafil as potent inhibitors of PDE5 in the management of erectile dysfunction has been documented recently (Jackson et al., 2005; Carson et al., 2005; Giuliano et al., 2005). However, due to lack of information other possible pharmacological mechanisms of these PDE5 inhibitors that may contribute to the proerectile potentials have not been explored clinically. Current study has demonstrated for the first time significant differences in the pharmacological profile of sildenafil, tadalafil and vardenafil in rabbit corpus cavernosum tissue strips.

Our results show that sildenafil, tadalafil and vardenafil were efficacious in decreasing the tone generated by noradrenaline. Tadalafil was the least potent among the 3 PDE5 inhibitors in reversing noradrenaline-induced tone. However, only sildenafil and vardenafil were capable of lowering the tone induced by K^+. The findings of sildenafil and vardenafil being more potent than tadalafil in cavernosal tissues precontracted with BAY K-8644, which was effectively inhibited by verapamil, further reaffirmed the differential calcium channel blocking activity of these PDE5 inhibitors. These observations, thus, suggest that sildenafil and vardenafil mediate relaxation by inhibiting transmembrane Ca^{2+} flux through both receptor-dependent

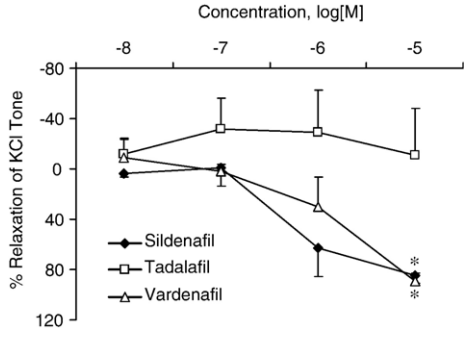

Fig. 6. Sildenafil and vardenafil evoked concentration-dependent relaxation of K^+-induced tone (120 mM KCl) in rabbit corpus cavernosum strips in vitro. Tadalafil was, however, relatively inert. Data are the mean±S.E.M. *$P<0.05$ relative to tadalafil.

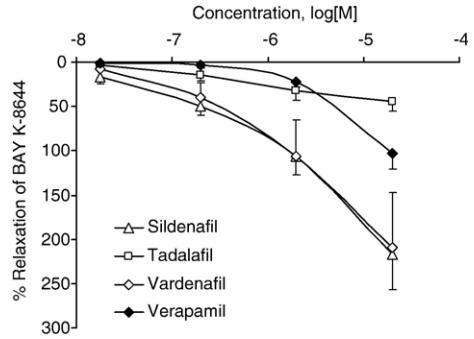

Fig. 7. Sildenafil, vardenafil, verapamil and tadalafil suppressed tonic contractions induced by BAY K-8644 in rabbit corpus cavernosum strips in vitro. Data are the mean±S.E.M.

and voltage-sensitive Ca^{2+} channels whereas tadalafil is more likely to inhibit receptor-operated Ca^{2+} channels in attaining cavernosal muscle relaxation. Differential effects of these PDE5 inhibitors were recently reported for rat aorta by Teixeira et al. (2006). Using a different pharmacological approach they demonstrated that vardenafil but not sildenafil or tadalafil induced vasorelaxation via blocking Ca^{2+} mobilization. This result clearly contrasts with our finding in which both vardenafil and sildenafil were potent muscle relaxants irrespective of the contractile agent used to induce tone in cavernosal tissue. One possible explanation for these discrepancies could be tissue dependent differences in Ca^{2+} involvement in rat aorta and rabbit corpus cavernosum.

The sensitivity of sildenafil, tadalafil and vardenafil to N^{ω}-nitro-L-arginine and ODQ suggested the contributory role of pre-existing endogenous NO–cGMP pathway. However, the incomplete inhibition of muscle relaxant effect by both ODQ and N^{ω}-nitro-L-arginine also shows that these PDE5 inhibitors facilitate CC muscle relaxation via an additional mechanism independent of classical NO–cGMP pathway; these findings are in agreement with previous reports on sildenafil and vardenafil in rabbit corpus cavernosum muscle (McAuley et al., 2001; Giuliano et al., 2003; Palea and Barras, 2003).

Among the 3 PDE5 inhibitors, tadalafil is known as a potent inhibitor of PDE11 that catalyzes the hydrolysis of both cAMP and cGMP (Bischoff, 2004b). However, it appears that the cAMP pathway is not involved in the relaxant action of not only tadalafil but also sildenafil and vardenafil since MDL12330A, a selective inhibitor of adenylate cyclase, did not significantly modify the concentration-related relaxation to all 3 PDE5 inhibitors in this study.

Sildenafil and vardenafil at higher concentrations inhibited the concentration response curve of noradrenaline in a non-competitive manner characterized by depression of maximal contraction. Hence, the relaxant/inhibitory effect of sildenafil and vardenafil in rabbit corpus cavernosum is unlikely to be mediated through antagonism of α-adrenoceptor. A recent report on rabbit isolated corpus cavernosum also indicated that sildenafil is not an α-adrenoceptor blocker (Palea and Barras, 2003). Nevertheless, these findings are suggestive of a risk of interaction with concomitant administration of α-blocker and high doses of sildenafil/vardenafil. Tadalafil, in this study, apparently does not intervene in the α-adrenoceptor mediated contractile mechanism of noradrenaline.

Although sildenafil is a selective PDE5 inhibitor preventing the destruction of cGMP, it has been shown to significantly increase cAMP in human corpus cavernosum tissues probably indirectly as a consequence of interaction between cGMP- and cAMP-mediated signal transduction mechanisms (Stief et al., 2000).

In addition to degradation by phosphodiesterase, cGMP is exported from cells by multidrug resistance protein 5 which is an adenosine triphosphate dependent export pump for cGMP. Co-expression of multidrug resistance protein 5 and PDE5 has been demonstrated in smooth muscle cells of the genitourinary system (Nies et al., 2002). PDE inhibitors including sildenafil and trequinsin potently inhibited multidrug resistance protein 5. Hence, binding of sildenafil to multidrug resistance protein 5 may constitute another pathway contributing to relaxation of corpus cavernosum muscle.

cGMP has been known to interact with protein kinase G which affects gap junctions and ion channels resulting in reduction in Ca^{2+} influx leading to a decrease in cytoplasmic Ca^{2+} levels, inactivation of myosin kinase and thus relaxation of smooth muscle cells. However, in human corpus cavernosum smooth muscle cell-attached patches sildenafil failed to activate the Ca^{2+}-activated K^{+} channels indicating that the direct relaxant response to sildenafil may not be due to opening of these ion channels (Lee and Kang, 2001).

Taken together, our results provide evidence suggesting that apart from their PDE5 inhibitory activity, sildenafil, tadalafil and vardenafil are capable of modulating Ca^{2+} mobilization leading to corpus cavernosum muscle relaxation. This inference is in line with our recent study on sildenafil in monkey cavernosum muscle (Lau et al., 2000a) and earlier report on the ability of calcium channel blockers such as verapamil and the dihydropyridines to reverse noradrenaline-induced contractions in rabbit cavernosal muscle in vitro (Kerfoot et al., 1993). The competencies of these PDE5 inhibitors in modulating Ca^{2+} entry leading to cavernosum muscle relaxation are compatible with their molecular structures as sildenafil and vardenafil differ only minimally while tadalafil differs markedly from sildenafil and vardenafil.

The ability of sildenafil to modulate Ca^{2+} mobilization may be considered as an alternative mechanism independent of the classical NO/cGMP pathway that could explain the proerectile effect of intracavernosal sildenafil in anesthetized rabbits, in the absence of pelvic nerve stimulation (McAuley et al., 2001). It is tempting to speculate that zaprinast, a PDE5 inhibitor that is structurally related to cGMP may share this mechanism as intracavernous administration in cat led to increased intracavernosal pressure and penile length (Doherty et al., 2001).

It is difficult to extrapolate in vitro findings obtained using isolated cavernosal tissue to normal physiologic response of intact cavernosal tissue owing to the different conditions such as absence of arterial flow, venous outflow, autonomic innervation and other homeostatic factors that might interfere with the cavernosal tone. In addition, concentrations of PDE5 inhibitors causing direct cavernosum muscle relaxation are notably higher than the estimated free peak plasma concentration range of 300 ng/ml, 400 ng/ml and 20 ng/ml after a single oral dose of sildenafil (100 mg), tadalafil (20 mg) and vardenafil (20 mg) respectively in man (Bischoff, 2004a). In view of species differences (Stief et al., 1998) current results warrant further confirmation in human corpus cavernosum.

References

Ballard, S.A., Gingell, C.J., Tang, K., Turner, L.A., Price, M.E., Naylor, A.M., 1998. Effects of sildenafil on the relaxation of human corpus cavernosum tissue in vitro and on the activities of cyclic nucleotide phosphodiesterase isozymes. J. Urol. 159, 2164–2171.

Bischoff, E., 2004a. Vardenafil preclinical trial data: potency, pharmacodynamics, pharmacokinetics, and adverse events. Int. J. Impot. Res. 16 (Suppl 1), S34–S37.

Bischoff, E., 2004b. Potency, selectivity, and consequences of nonselectivity of PDE inhibition. Int. J. Impot. Res. 16 (Suppl 1), S11–S14.

Carson, C., Shabsigh, R., Segal, S., Murphy, A., Fredlund, P., Kuepfer, C., 2005. Trial Evaluating the Activity of Tadalafil for Erectile Dysfunction–United States (TREATED–US) Study Group, Efficacy, safety, and treatment satisfaction of tadalafil versus placebo in patients with erectile dysfunction evaluated at tertiary-care academic centers. Urology 65, 353–359.

Doherty, P.C., Bivalacqua, T.J., Champion, H.C., Kadowitz, P.J., Greenwood-Van Meerveld, B., Berzetei-Gurske, I., Hellstrom, W.J., 2001. Direct effects of selective type 5 phosphodiesterase inhibitors alone or with other vasodilators on the erectile response in cats. J. Urol. 165, 1004–1009.

Fawcett, L., Baxendale, R., Stacey, P., McGrouther, C., Harrow, I., Soderling, S., Hetman, J., Beavo, J.A., Phillips, S.C., 2000. Molecular cloning and characterization of a distinct human phosphodiesterase gene family: PDE11A. Proc. Natl. Acad. Sci. U. S. A. 97, 3702–3707.

Francis, S.H., Turko, I.V., Corbin, J.D., 2001. Cyclic nucleotide phosphodiesterases: relating structure and function. Prog. Nucleic Acid Res. Mol. Biol. 65, 1–52.

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.

Giuliano, F., Donatucci, C., Montorsi, F., Auerbach, S., Karlin, G., Norenberg, C., Homering, M., Segerson, T., Eardley, I., Vardenafil Study Group, 2005. Vardenafil is effective and well-tolerated for treating erectile dysfunction in a broad population of men, irrespective of age. BJU Int. 95, 110–116.

Jackson, G., Gillies, H., Osterloh, I., 2005. Past, present, and future: a 7-year update of Viagra (sildenafil citrate). Int. J. Clin. Pract. 59, 680–691.

Kerfoot, W.W., Park, H.Y., Schwartz, L.B., Hagen, P.O., Carson III, C.C., 1993. Characterization of calcium channel blocker induced smooth muscle relaxation using a model of isolated corpus cavernosum. J. Urol. 150, 249–252.

Lau, L.C., Adaikan, P.G., Ng, S.C., 2000a. Inhibition by phosphodiesterase inhibitors of excitatory neurotransmission in monkey is different from that in human corpus cavernosum. 9th World Meeting on Impotence Research, 26–30 Nov 2000, Perth Western Australia, Abstract M97.

Lau, L.C., Adaikan, P.G., Ratnam, S.S., Ng, S.C., 2000b. Inhibition of adrenergic neurotransmission in the cavernosum — an additional mechanism of action. Int. J. Impot. Res. 12 (Suppl 2), S17.

Lee, S.W., Kang, T.M., 2001. Effects of nitric oxide on the Ca2+-activated potassium channels in smooth muscle cells of the human corpus cavernosum. Urol. Res. 29, 359–365.

McAuley, I.W., Kim, N.N., Min, K., Goldstein, I., Traish, A.M., 2001. Intracavernosal sildenafil facilitates penile erection independent of the nitric oxide pathway. J. Androl. 22, 623–628.

Nies, A.T., Spring, H., Thon, W.F., Keppler, D., Jedlitschky, G., 2002. Immunolocalization of multidrug resistance protein 5 in the human genitourinary system. J. Urol. 167, 2271–2275.

Palea, S., Barras, M., 2003. Comparison of the relaxant effects of alfuzosin, phentolamine and sildenafil on rabbit isolated corpus cavernosum. BJU Int. 91, 873–877.

Stief, C.G., Uckert, S., Becker, A.J., Truss, M.C., Jonas, U., 1998. The effect of the specific phosphodiesterase (PDE) inhibitors on human and rabbit cavernous tissue in vitro and in vivo. J. Urol. 159, 1390–1393.

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.

Teixeira, C.E., Priviero, F.B., Webb, R.C., 2006. Differential effects of the phosphodiesterase type 5 inhibitors sildenafil, vardenafil, and tadalafil in rat aorta. J. Pharmacol. Exp. Ther. 316, 654–661.

Uckert, S., Hedlund, P., Waldkirch, E., Sohn, M., Jonas, U., Andersson, K.E., Stief, C.G., 2004. Interactions between cGMP- and cAMP-pathways are involved in the regulation of penile smooth muscle tone. World J. Urol. 22, 261–266.

Usowicz, M.M., Gigg, M., Jones, L.M., Cheung, C.W., Hartley, S.A., 1995. Allosteric interactions at L-type calcium channels between FPL 64176 and the enantiomers of the dihydropyridine Bay K 8644. J. Pharmacol. Exp. Ther. 275, 638–645.

Wallis, R.M., Corbin, J.D., Francis, S.H., Ellis, P., 1999. Tissue distribution of phosphodiesterase families and the effects of sildenafil on tissue cyclic nucleotides, platelet function and the contractile responses of trabeculae carneae and aortic rings in vitro. Am. J. Cardiol. 83, 3C–12C.