0013-7227/07/$15.00/0
Printed in U.S.A.

Endocrinology 148(3):1019–1029

doi: 10.1210/en.2006-1079

Characterization and Functional Role of Androgen-Dependent PDE5 Activity in the Bladder

Sandra Filippi, Annamaria Morelli, Peter Sandner, Benedetta Fibbi, Rosa Mancina, Mirca Marini, Mauro Gacci, Linda Vignozzi, Gabriella Barbara Vannelli, Marco Carini, Gianni Forti, and Mario Maggi

Interdepartmental Laboratory of Functional and Cellular Pharmacology of Reproduction, Departments of Pharmacology and Clinical Physiopathology (S.F.), Andrology (A.M., B.F., R.M., L.V., Ma.M.) and Endocrinology Unit (G.F.), Department of Physiopathology, Center for Research, Transfer and High Education DENOTHE, Department of Anatomy, Histology and Forensic Medicine (Mi.M., G.B.V.), Department of Urology (M.G., M.C.), University of Florence, 50139 Florence, Italy; and Bayer Health Care (P.S.), Pharma Research EU, D-42096 Wuppertal, Germany

Benign prostate hyperplasia is the most common disease in the aging male, often comorbid with erectile dysfunction. Phosphodiesterase type 5 (PDE5) inhibitors (sildenafil, tadalafil, and vardenafil) decrease lower urinary tract symptoms in patients with erectile dysfunction and BPH. We studied PDE5 expression and activity in the human bladder and PDE5i effects both *in vitro* (human and rat) and *in vivo* (rat). PDE5 is highly expressed in rat and human bladder and immunolocalized in vascular endothelium and muscle fibers. Sildenafil, tadalafil, and vardenafil blocked 70% of the total cGMP-catabolizing activity; vardenafil was the most potent (IC_{50} = 0.3 nM). In human bladder cells and in rat strips, a PDE-resistant cGMP analog, SP-8-Br-PET-cGMPS, induced, respectively, a consistent antiproliferative and relaxant effect. In contrast, the nitric oxide donor sodium nitroprusside (SNP) was almost ineffective. However, blocking PDE5 with vardenafil increased SNP antiproliferative and relaxant activity up to the level observed with SP-8-Br-PET-cGMPS. We also found that castration decreased, and T supplementation restored, PDE5 gene expression in rat bladder. Accordingly, bladder strips from castrated rats were more sensitive to SNP-induced relaxation than strips from control or T-replaced rats, whereas in the presence of vardenafil, all groups showed the same SNP sensitivity. To discover whether vardenafil affects bladder activity *in vivo*, the rat bladder outlet obstruction model was used. Chronic treatment with 10 mg/kg·d vardenafil significantly reduced nonvoiding contractions (47%, $P < 0.05$ *vs.* placebo) up to tamsulosin level (51%). Overall, these results demonstrate that PDE5 regulates bladder smooth muscle tone, strongly limiting the nitric oxide/cGMP signaling, and that vardenafil, by blocking PDE5, may be a possible therapeutic option for bladder dysfunction by ameliorating irritative lower urinary tract symptoms. (*Endocrinology* 148: 1019–1029, 2007)

BENIGN PROSTATE HYPERPLASIA (BPH) is the most common and costly urinary disorder affecting the aging male. It is clinically characterized by the progressive and bothersome development of lower urinary tract symptoms (LUTS, see Refs. 1 and 2 for reviews). Essentially, the hyperplastic prostate, by compressing the urethra, decreases the bladder outlet and, therefore, urine outflow. In the earliest stages, this urethral obstruction is compensated for by bladder wall hypertrophy associated with increased contractile activity. Subsequently, the hypertrophic bladder becomes dysfunctional with the occurrence of nonvoiding contractions (NVCs) and finally it decompensates with more frequent NVCs during the filling phase and deterioration of the ability to generate adequate voiding pressure. BPH generates LUTS partly because of prostate overgrowth and flow obstruction (static component) and partly because of bladder decompensation and overactivity (dynamic component). The static component is mostly responsible for obstructive symptoms such as weak stream, intermittent urinary flow, and/or straining to void. The dynamic component is responsible for the occurrence of irritating symptoms such as urinary frequency, urgency, and nocturia (1). Alteration in central and peripheral nervous system control of the bladder contraction and voiding cycle can also cause bladder dysfunction and LUTS.

To avoid the progressive deterioration of the vesicoprostatic complex, several compounds have been developed; α-adrenergic antagonists and 5α-reductase inhibitors represent the most current effective treatment options (2).

Erectile dysfunction (ED) is another common disorder of elderly males and is often comorbid with BPH (3). In the last few years, some nonplacebo-controlled reports indicate that treatment of patients with ED with phosphodiesterase type 5 inhibitors (PDE5i) such as sildenafil improves not only ED symptoms, but also LUTS (4–6). Preliminary results from double-blind, placebo-controlled studies with sildenafil (7, 8) and tadalafil (9) seem to confirm the positive effect of PDE5i in LUTS. Because previous studies reported that the human bladder expresses PDE5 gene (10, 11) and protein (11), we initiated studies to fully characterize PDE5 in the bladder and investigate its physiological significance for the treatment of LUTS.

Materials and Methods

Rat and human tissue collection

Male Sprague Dawley rats (275–300 g) were purchased from Harlan Italy (San Pietro al Natisone, Udine, Italy). A subset of male rats was

First Published Online November 30, 2006

Abbreviations: BOO, Bladder outlet obstruction; BPH, benign prostate hyperplasia; ED, erectile dysfunction; ET-1, endothelin-1; hBC, human bladder smooth muscle cells; LUTS, lower urinary tract symptoms; NVC, nonvoiding contraction; PDE5, phosphodiesterase type 5; PDE5i, phosphodiesterase type 5 inhibitor; SNP, sodium nitroprusside; T, testosterone.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

further separated into three groups: 1) control, 2) surgical castration, and 3) castration replaced by testosterone (T, testosterone propionate; Schering, Milan, Italy; 30 mg/kg·wk sc) (12). Castration was performed through the scrotal route under sodium pentobarbital anesthesia (45 mg/kg ip). Two wk after surgery and 1 wk after the last supplementation of T, rats were killed by cervical dislocation and blood was drawn from the heart for T measurements. The urinary bladders were exposed, removed, and cleaned of connective tissue and adherent fat. The lowest half of the bladder was longitudinally bisected and used for *in vitro* studies. The urothelium–suburothelium was kept intact. Seminal vesicles and ventral prostate were also separated, dried, and weighed.

Rat experiments were performed according to the Italian Ministerial Law no. 116/92 and approved by the Institutional Animal Care and Use Committee of the University of Florence, Florence, Italy.

Human bladder neck samples were derived from male patients (n = 6, age range 55–80 yr, mean ± SD 71.2 ± 9.2 yr) undergoing transvesical prostatectomy for large BPH (n = 2) or radical cystectomy for invasive transvesical cell carcinoma localized far from the bladder trigone (n = 4). Other human tissues from the male urogenital tract (testis, vas deferens, epididymis, corpora cavernosa, prostate, and kidney) were collected during surgery for benign diseases. All tissue samples were obtained after the approval of the Hospital Committee for Investigation in Humans (Azienda Ospedaliero Universitaria Careggi, Florence, Italy; Protocol no. 6783-04) and after receiving consent from the informed patients. Immediately after removal, tissue samples were shock frozen in liquid nitrogen and stored at −80 C until RNA preparation or enzymatic assays.

Measurement of testosterone

T plasma levels were measured using an Automated Chemiluminescence System (Bayer Diagnostics, East Walpole, MA) as previously described (11).

Contractility studies

Contractility studies were carried out as previously described (13). Briefly, rat and human bladder strips were vertically mounted under 1.0 g resting tension in organ chambers. This resting tension was optimal for both rat and human strips. Preparations were aerated with 95% O_2 and 5% CO_2 and bathed in a physiological salt solution of the following composition (mM): NaCl 119, KCl 4.7, $NAHCO_3$ 15, KH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 1.5, and glucose 11.1. Changes in isometric tension were recorded on a chart polygraph (Battaglia Rangoni, San Giorgio di Piano, Bologna, Italy). In human preparations, high potassium salt solution, made by equimolar substitution of sodium by potassium, increased the tonic tension with the maximum effect obtained at 80 mM. The high potassium solution had the following composition (mM): NaCl 133.5, KCl 80, $NAHCO_3$ 6, KH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 1.5, and glucose 11.1. The KCl-induced increase in tension value was taken as 100% and the increase recorded in the presence of different concentrations of various agonists [endothelin-1 (ET-1); Calbiochem, San Diego, CA; and carbachol; Sigma-Aldrich, St. Louis, MO] referred to this value. Responsiveness to contractile agents was not affected by previous exposure to high potassium solution (data not shown).

The relaxant effect induced by different drugs [sodium nitroprusside (SNP); Sigma-Aldrich; Sp-8-Br-PET-cGMPS (Biolog, Hayward, CA)] and the selected PDE5 inhibitors (vardenafil, sildenafil, and tadalafil) was evaluated in bladder strips precontracted with carbachol (10 μM). In some experiments, dose–response curves for PDE5i-induced relaxation were conducted in the presence of a fixed concentration of SNP (300 nM). In other experiments, the relaxant effect of increasing concentrations of SNP was evaluated in the presence or absence of a fixed concentration of vardenafil (100 nM). Stock solutions of sildenafil were made in ethanol; stock solutions of Sp-8-Br-PET-cGMPS were made in dimethylsulfoxide; stock solutions of carbachol, vardenafil, taladafil, and SNP were dissolved daily in double-distilled water; and further dilutions of all substances were made in Krebs' solution. Control experiments showed that the concentrations of dimethylsulfoxide or ethanol used neither modified the relaxation induced by the agents nor the vasoconstrictor response to carbachol.

Cell cultures

Primary human bladder smooth muscle cells (hBC) were obtained and cultured as previously described (14). Different cultures were used after five to 12 passages. For the cell proliferation assay, 2.3×10^4 hBC were seeded onto 12-well plates in their growth medium. After 24 h, the cells were washed in PBS, incubated overnight in phenol red- and serum-free medium, and treated with increasing concentrations (0.1 nM–10 μM) of SNP and Sp-8-Br-PET-cGMPS for 48 h with or without a fixed concentration (1 nM) of vardenafil. In the other set of experiments, increasing concentrations (0.1 pM–1 μM) of vardenafil were added for 48 h to a fixed concentration (1 μM) of SNP. For each experiment, the medium was changed and stimuli were repeated after 24 h. Cells in phenol red- and serum-free medium containing 0.1% BSA were used as controls. After 48 h, cells were trypsinized, and each experimental point was derived from hemocytometer counting and then averaging of nine different fields for each well. The experiments were performed four times using two hBC preparations, each derived from different patients. Each experimental point was repeated in duplicate or triplicate. Results are expressed as the percentage variation (mean ± SEM) from control. Responsiveness to SNP did not change among different passages (not shown).

Real-time quantitative RT-PCR

Isolation of RNA from rat and human tissues was performed using RNAeasy kit (Qiagen, Valencia, CA). The mRNA quantitative analysis was performed according to the fluorescent TaqMan methodology following previously described protocols (11, 15). PCR primers and probes were purchased from Applied Biosystems (Foster City, CA). Amplification and detection were performed using ABI Prism 7700 Sequence Detection. Each measurement was carried out in duplicate. Analysis of rat tissue was performed by relative quantitation based on the comparative Ct method and using the β-2 microglobulin as reference gene as already reported (15). The comparative Ct method uses arithmetic formulas to obtain quantitative values from the threshold cycle (Ct) number at which the increase in fluorescent signal, associated with an exponential increase in PCR products, crosses a given threshold. The maximum change in Ct values of the sample (ΔCt_{sample}) was determined by subtracting the average of duplicate Ct values of the reference gene from the average of duplicate Ct values of the target gene. Analysis of human tissues was performed by absolute quantitation using an external standard curve obtained as previously described (11). Known starting concentrations of PDE5 standard cDNA, ranging from 3.5×10^6 to 3.5×10^1 copies, were used to construct the external calibration curve. Results are expressed as PDE5 mRNA molecules per microgram total RNA.

Phosphodiesterase activity assay

Human bladder tissues were homogenized using an Ultra-Turrax (5:1, vol:wt) as previously reported (11). The homogenates were aliquoted and stored at −80 C until use. Protein concentration was determined by the Bradford method (16). PDE activity for human samples was carried out as described by Morelli *et al.* (11). Briefly, for IC_{50} determination, protein aliquots of 0.02 mg of human bladder homogenate were incubated in a final vol of 200 μl for 5 min at 30 C with 0.5 μM cold cGMP and 0.1 μM [^{3}H]-cGMP in 40 mM 4-morpholinopropane sulfonic acid buffer (pH 7.0) containing 1 mM EDTA, 0.8 mM EGTA, 5 mM Mg acetate, 0.2 mg/ml BSA with or without inhibitors. The inhibitors used to characterize PDE activity were vardenafil (from 10^{-12} to 10^{-6} M), tadalafil and sildenafil (from 10^{-11} to 10^{-6} M), zaprinast and EHNA (from 10^{-9} to 10^{-3} M), dipyridamole, cilostamide, and vinpocetine (from 10^{-9} to 10^{-4} M). Reactions were terminated by incubation at 100 C for 1 min. Aliquots of 60 μl of each sample were applied to 60 F_{254} silica-gel plates using absolute ethanol/H_2O (70:30, vol:vol) as eluant to separate cGMP, GMP, and guanine. cGMP, GMP, and guanine lanes, after visualization under UV light, were scraped; silica was extracted with 1 ml H_2O, and radioactivity was measured in InstaGel Plus by a β-counter instrument. The enzymatic activity was evaluated as percentage of substrate into product conversion as follows: conversion (%) = [products count/(substrate + products counts)] × 100. The total recovery of cGMP, GMP, and guanine was 95% to 100%.

Immunohistochemistry

Immunohistochemical studies were performed as previously described (11) on deparaffinized rehydrated sections of human bladder tissues. Briefly, the slides were stained for indirect immunoperoxidase technique using polyclonal anti-PDE5 (1:100 vol/vol) (17) as primary antibody. Controls were performed omitting the primary antibody (11).

Bladder outlet obstruction

In vivo experiments in rats were performed according to the German Law for the Protection of Laboratory Animals and were conducted according to the approved guidelines of the local Laboratory Animals Science & Welfare council. Experiments were performed with female Sprague Dawley rats (Harlan Winkelmann, Borchen, Germany) with a body weight between 200 and 250 g. For the bladder outlet obstruction (BOO), rats were anesthetized and the urethra was partially ligated (A 1.0 mm) by a silk ligature. Twenty-four hours after surgery, rats were divided into four groups and were treated with placebo (group 1), 10 μg/kg·d tamsulosin (group 2), 3 mg/kg·d vardenafil (group 3), and 10 mg/kg vardenafil (group 4). Tamsulosin, a uroselective α (1A)-adrenergic receptor antagonist, was isolated from FLOMAX tablets and vardenafil was supplied by Bayer Health Care (Wuppertal, Germany). The compounds were administered in standard rat chow homogenously supplemented with the compounds supplied by Sniff (Spezialdiäten GmbH, Soest, Germany). Rats were kept for 5 wk on placebo, tamsulosin, or vardenafil rat chow and had free access to tap water.

Conscious cystometry

Within the sixth week after BOO surgery and onset of treatment, a conscious cystometry was performed. Twenty-four hours before the cystometry, a polyethylene catheter (PE50) was implanted into the bladder dome and tunneled sc to the back neck of the animal. After this surgery, animals fasted but had free access to tap water. The animals were placed in a Ballman's cage for cystometry. After 1 h of recovery, the bladder catheter was connected to a pressure transducer for measurement of intravesical pressure (MLT0698; AD Instruments, Colorado Springs, CO) and to an infusion pump for continuous infusion of saline solution at a flow rate of 10 ml/h. All bladder contractions 5 cmH_2O or more (4 mm Hg), which did not result in urinary outflow, were counted as NVCs. The efficacy of treatment was quantified through calculation of the NVCs per micturition interval.

Statistical analysis

Results are expressed as mean ± SEM for n experiments. Statistical analysis was performed with one-way ANOVA or Student's *t* test for paired or unpaired data, whenever appropriate; $P < 0.05$ was considered significant. EC_{50} and IC_{50}, maximal stimulation (E_{max}), and maximal inhibition (I_{max}) values were calculated using the computer program ALLFIT (18). The same program was used to statistically analyze differences in sigmoidal dose-response curves (18).

Results

Figure 1 shows the expression of PDE5 mRNA in the

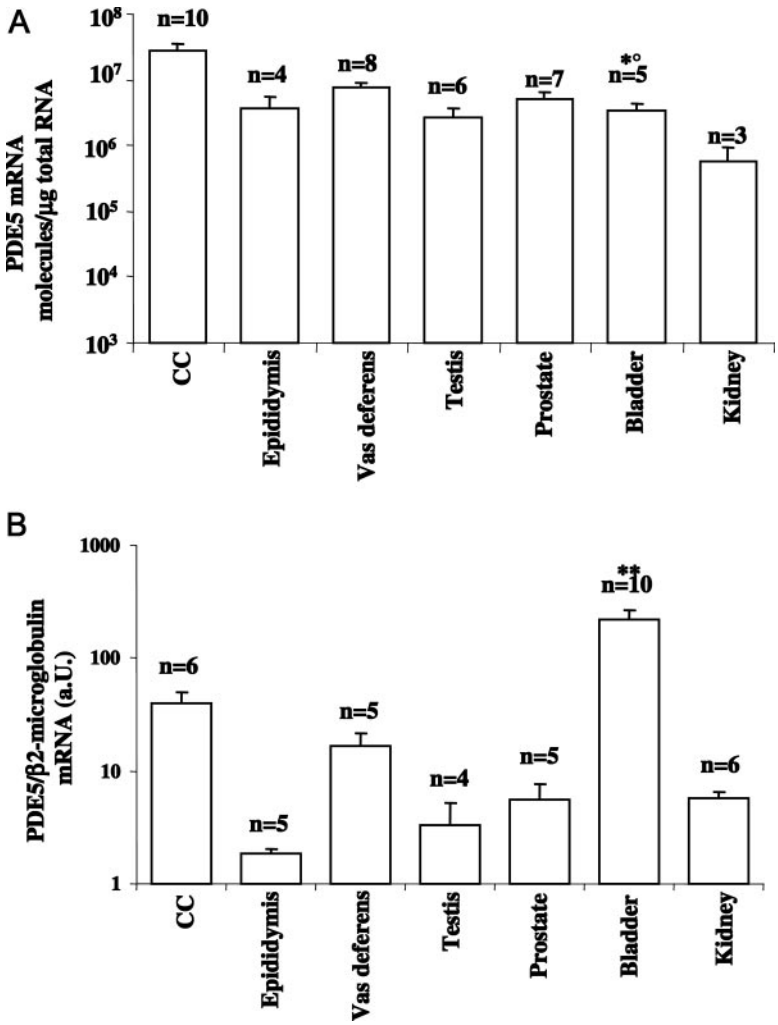

FIG. 1. Quantitative expression, by real-time RT-PCR, of PDE5 mRNA in (A) human and (B) rat male urogenital tissues. A, Data are expressed as PDE5 mRNA molecules/μg total RNA ± SEM obtained according to a standard curve method for absolute quantitation. B, Data are expressed as mean ± SEM of arbitrary units (a.U.) calculated according to the comparative Ct method and using the β_2-microglobulin as reference gene for normalization. n, number of analyzed samples. *, $P < 0.05$ *vs.* human kidney; °, $P < 0.001$ *vs.* human corpus cavernosum (CC); **, $P, < 0.01$ *vs.* rat corpus cavernosum (CC).

human (panel A) and rat (panel B) urogenital tract. The PDE5 gene is expressed similarly to other portions of the male genital tract ($3.5 \pm 1 \times 10^6$ molecules/μg total RNA) in the human bladder but is more abundant than in the kidney ($6.0 \pm 3.5 \times 10^5$ molecules/μg total RNA, $P < 0.05$). However, human bladder PDE5 gene expression it is still 10-fold lower than in the corpora cavernosa ($2.8 \pm 0.7 \times 10^7$ molecules/μg total RNA, $P < 0.001$). Conversely, PDE5 expression is even higher in the rat bladder than in the corpus cavernosum ($P < 0.01$).

To verify whether typical PDE5 enzymatic activity corresponded to this gene expression, we evaluated the ability of different PDE inhibitors to antagonize cGMP conversion to metabolites in human bladder homogenates using a previously reported experimental assay (11, 13, 19, 20). Results were compared with those obtained in human corpora cavernosa (19, 20). Specific PDE5 inhibitors, sildenafil, tadalafil, and vardenafil, antagonized cGMP metabolism at the lowest IC_{50} values (in the nanomolar range; Fig. 2A and Table 1) in the bladder. In addition, IC_{50} values for these compounds are identical in both human corpora cavernosa and bladder tissues with vardenafil being the most potent (Fig. 2B). In fact, vardenafil IC_{50} is at least one log unit lower than IC_{50} values of sildenafil and tadalafil ($P < 0.02$, see Table 1), which were not statistically different between each other ($P = 0.146$, see Table 1) in the human bladder. Overall, sildenafil, tadalafil, and vardenafil inhibit 70 ± 15% of the total cGMP metabolism (Fig. 2A). The other two PDE5i tested, dipyridamole and zaprinast, show IC_{50} values at least three orders of magnitude higher than sildenafil, tadalafil, and vardenafil, which reflect their described activity on PDE5 enzyme (21, 22). Interestingly, in contrast to the selective PDE5i, they inhibited almost all cGMP conversion to metabolites. According to PDE5 gene expression, vardenafil-sensitive cGMP breakdown activity was higher in the penis than in the bladder

TABLE 1. IC_{50} values of different PDE inhibitors in human bladder homogenates

	IC_{50} nM		IC_{50} μM
Vardenafil	0.3 ± 0.2	Dipyridamole	10.9 ± 2.7
Sildenafil	3.2 ± 2.4	Zaprinast	8.8 ± 2.2
Tadalafil	6.07 ± 4.5	Cilostamide	40.5 ± 10.7
		EHNA	209 ± 184
		Vinpocetine	>1000

Values are derived from simultaneous fitting with ALLFIT (18) of a family of inhibition curves of cGMP conversion to metabolites in human bladder homogenates with the indicated PDE inhibitors.

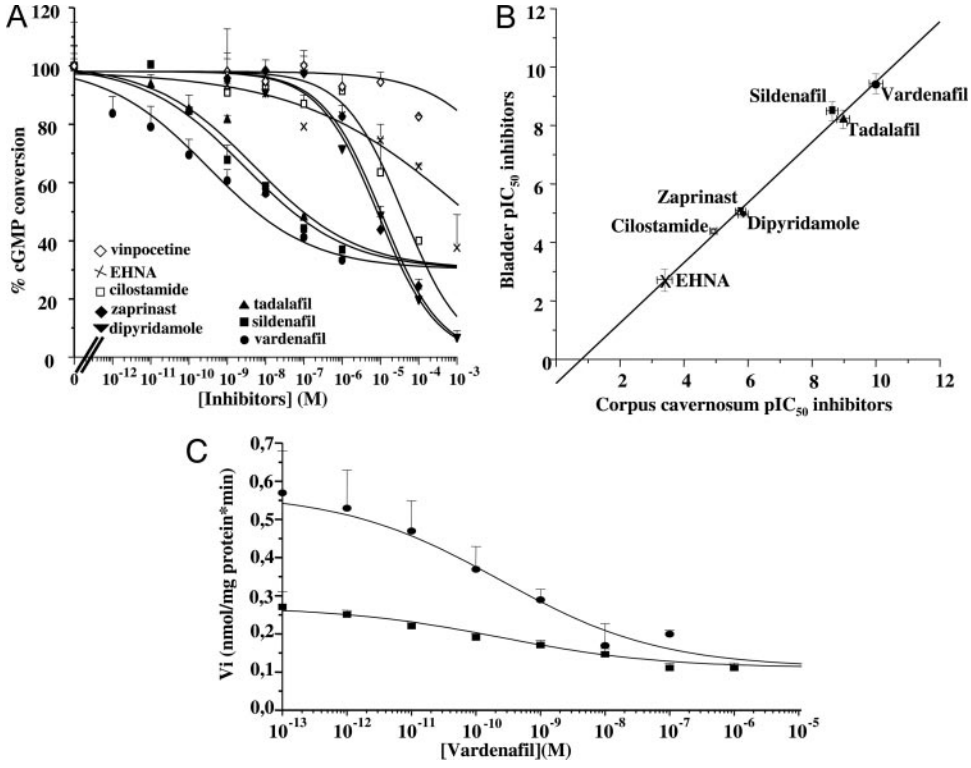

FIG. 2. Characterization of cGMP-PDE activity in human bladder homogenates. A, cGMP breakdown in human bladder tissue extracts were evaluated in the presence of the following inhibitors: vinpocetine (*open diamonds*) for PDE1, EHNA (*stars*) for PDE2, cilostamide (*open squares*) for PDE3 and tadalafil (*upward closed triangles*), sildenafil (*closed squares*), and vardenafil (*closed circles*) for PDE5. Results obtained with zaprinast (selective for PDE5, PDE6, and PDE9, *closed diamonds*) and dipyridamole (selective for PDE5, PDE6 and PDE10, *downward closed triangles*) are also shown. B, Linear relationship ($r = 0.99$) between $-\log IC_{50}$ vaules (pIC_{50}–pIC_{50} plot) of different PDE inhibitors in the human corpus cavernosum (CC) (abscissa) *vs.* the human bladder (ordinate). Data (± SEM) were derived from ALLFIT (18) analysis of sigmoidal inhibition curves of cGMP breakdown, as shown in panel A. *Symbols* are as in panel A. Results obtained with vinpocetine were omitted because of the negligible inhibition in both tissues. Results from human corpora cavernosa were obtained from previous reports (19, 20). C, Vardenafil-sensitive cGMP breakdown activity in human CC (*closed circle*) and bladder (*closed squares*). *Ordinate*: cGMP hydrolyzing activity expressed as Vi (initial velocity, nmol/mg protein*min). *Abscissa*: vardenafil concentration (M).

(Fig. 2C), although IC_{50} values for vardenafil were not different in the two tissues (shared IC_{50} = 0.24 ± 0.12 nM).

Immunocytochemistry was performed to localize PDE5 expression in the human bladder. The results clearly show that urothelium is negative, whereas immunolabeling was restricted to endothelial and smooth muscle cells of the blood vessels and to smooth muscle cells of muscle bundles. Typical examples are shown in Figure 3.

Because PDE5 is mostly localized in smooth muscle fibers and vardenafil was shown to be the most potent inhibitor of cGMP breakdown, we next studied the effect of this selective PDE5i on human bladder contractility. The effect of two distinct well-known bladder contractile agents, ET-1 and carbachol (2), was first studied in isolated human bladder strips. As shown in Fig. 4A, the maximum efficacy of carbachol in stimulating human bladder contraction was threefold higher than ET-1 (carbachol E_{max} = 218 ± 0.5%; ET-1 E_{max} = 72.9 ± 0.5%, $P < 0.0001$), but carbachol potency was at least 15 times lower than that of ET-1 (carbachol EC_{50} = 359 ± 3.5 nM; ET-1 EC_{50} = 24.5 ± 1.1 nM, $P < 0.0001$). In carbachol (10 μM) precontracted strips, the nitric oxide donor, SNP, partially relaxed the human preparations with IC_{50} = 137 ± 69 nM and I_{max} = 36.5 ± 3.9% (Fig. 4B). Preincubation with vardenafil (100 nM) significantly ($P < 0.0001$) increased SNP-induced relaxation (I_{max} = 62.3 ± 2.5%) without altering the relative IC_{50} (Fig. 4B).

Figure 5 and Table 2 show the effect of exposure to increasing concentrations of SNP in hBC (13, 14). Growth inhibition obtained with SNP was time- and dose-dependent (see in Table 2 and Fig. 5A, respectively). Time course experiments indicate that a significant inhibition with SNP (1 μM) was obtained only after 48-h exposure to the nitric oxide donor (Table 2). However, even at this time point, inhibition of bladder cell proliferation was negligible: less than 25% at the maximal concentration of SNP tested (10 μM, Fig. 5A). Simultaneous incubation for 48 h with increasing concentrations of SNP and a fixed concentration of vardenafil (1 nM) significantly increased the antiproliferative effect of SNP at all SNP concentrations tested (Fig. 5A). In the presence of vardenafil, maximal SNP-induced growth inhibition reached almost 60%. Similar antiproliferative activity was obtained with a PDE-resistant, cGMP analog: SP-8-Br-PET-cGMPS (Fig. 5B). In this case, the effect of SP-8-Br-PET-cGMPS was not potentiated by vardenafil (1 nM) according to the PDE-resistant characteristic of the compound (Fig. 5B). Figure 5C shows the effect of increasing concentrations of vardenafil (0.01 pM–1 μM) on growth inhibition induced by a submaximal (1 μM) concentration of SNP. In this experimental condition, vardenafil potentiated the antiproliferative effect of SNP with an IC_{50} = 0.72 ± 0.6 nM, which is in close agreement with its IC_{50} on cGMP-dependent PDE activity (Fig. 2; Table 1).

Figure 6A shows the effect of increasing concentrations of SNP and of a PDE-resistant, cGMP analog, SP-8-Br-PET-cGMPS, in carbachol-precontracted rat bladder strips. As observed for human bladder (see previously), SNP only marginally relaxed rat bladder strips (I_{max} = 32 ± 2.1%, IC_{50} = 2.34 ± 1.7 μM). This relaxation was significantly ($P < 0.0001$) lower than that obtained with the PDE-resistant cGMP analog (I_{max} = 70.1 ± 26.5%), suggesting the presence of a cGMP-metabolizing activity also in the rat bladder. Therefore, we tested the effect of different PDE5i (vardenafil, tadalafil, and sildenafil) on carbachol-precontracted strips (Fig. 6B). At high concentrations (micromolar range), all three PDE5i relaxed the bladder preparations. Although sildenafil and tadalafil were equipotent in inducing bladder relaxation (shared IC_{50} = 172 ± 69 μM), vardenafil was almost two orders of magnitude more potent (IC_{50} = 5.1 ± 1.2 μM, $P < 0.0001$ *vs.* sildenafil and tadalafil). Preincubation with a submaximal concentration of the nitric oxide donor

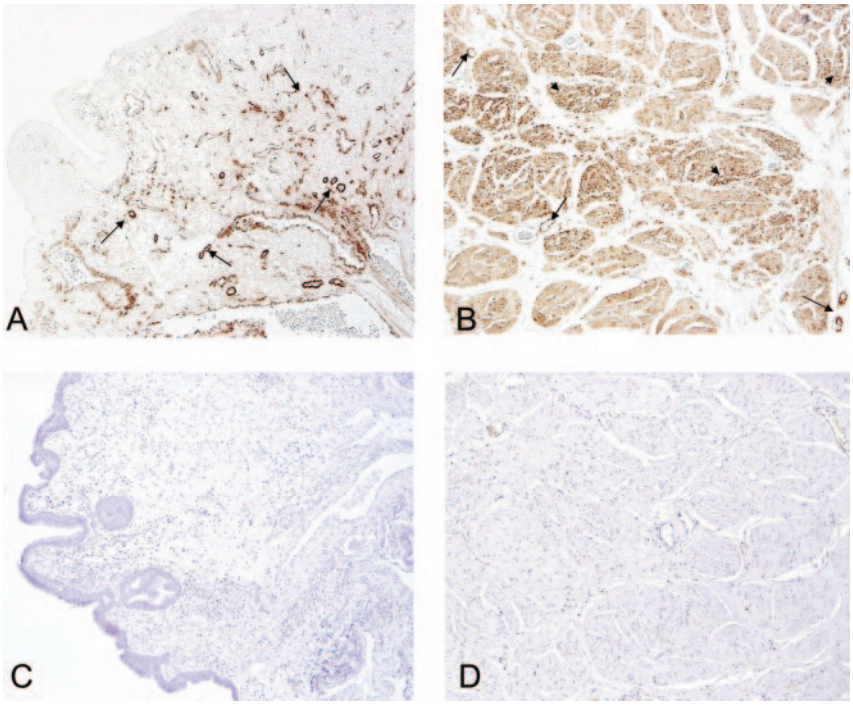

FIG. 3. Immunolocalization of PDE5 in the human bladder. Transverse sections through the human urinary bladder wall stained with a polyclonal antibody against PDE5. A, PDE5-immunopositive cells are localized in the connective tissue of the mucosa. In particular, endothelial and smooth muscle cells (*arrows*) of the blood vessels are stained. The folded transitional epithelium is unstained (magnification, ×40). B, The muscular fibers (*arrowheads*) show an intense immunostaining for PDE5. The blood vessels (*arrows*) are also immunopositive (magnification, ×40). C and D, Control section obtained by omitting the primary PDE5 antibody and counterstained with hematoxylin (magnification, ×40).

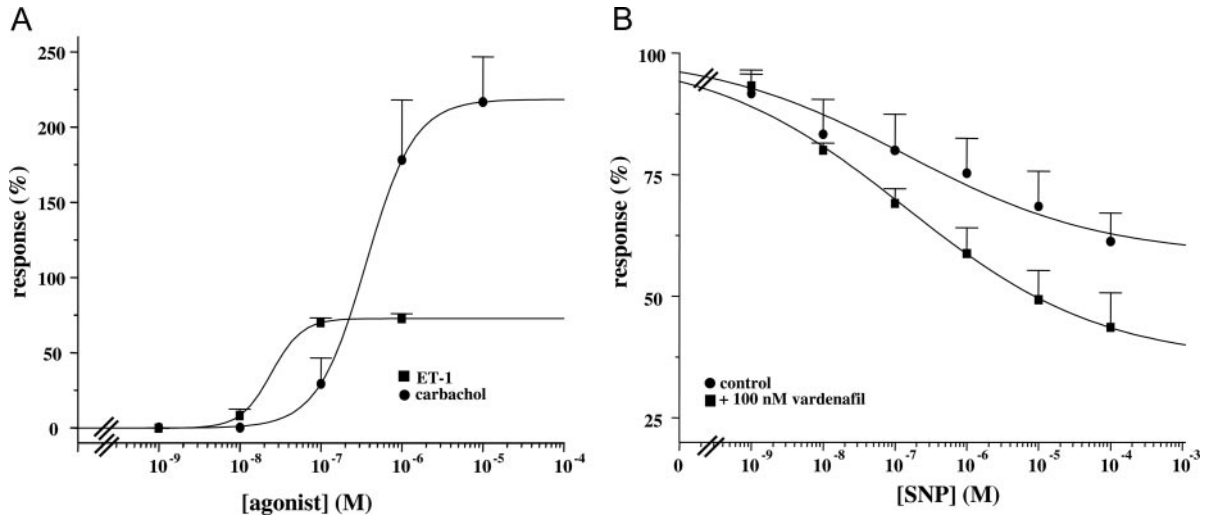

FIG. 4. Effect of vardenafil on human bladder contractility. A, Effect of increasing concentrations of ET-1 (*closed squares*, n = 3 in three separate experiments) and carbachol (*closed circles*, n = 4 in four separate experiments) *in vitro* contractility of human bladder strips. *Ordinate*: contractile activity expressed as percentage of the maximal response obtained with potassium chloride (80 mmol/liter). *Abscissa*: concentration of the agonist. Data are expressed as the mean ± SEM. The relative EC_{50} values are reported in the text. B, Concentration-response curve for SNP in human bladder strips in the absence (*closed circles*, n = 5 in five separate experiments) or presence (*closed squares*, n = 4 in four separate experiments) of 100 nM vardenafil. *Ordinate*: contractile tone induced by carbachol (10 μM). Maximal response to carbachol, before the addition of SNP, was taken as 100%. The effect of the relaxant agents was evaluated as a percentage of this response. *Abscissa*: SNP concentration (M). Each *point* represents mean ± SEM. The relative EC_{50} values are reported in the text.

SNP (0.3 μM) significantly shifted to the left PDE5i dose–response curves ($P < 0.05$, Fig. 6B). Also in this experimental condition, vardenafil was the most potent ($IC_{50} = 0.4 \pm 0.06$ μM, $P < 0.0001$ *vs.* sildenafil and tadalafil). As previously observed, IC_{50} values for sildenafil and tadalafil were not significantly different (shared $IC_{50} = 27.7 \pm 9.2$ μM). Preincubation of bladder strips with a fixed concentration of vardenafil (100 nM), which *per se* only marginally relaxes bladder preparations (see Fig. 6B), significantly ($P < 0.0001$) increased SNP relaxant effect to an extent not significantly different from SP-8-Br-PET-cGMPS ($P = 0.516$, Fig. 6A).

Figure 6 also shows results obtained in rat bladder, in which the androgen milieu was manipulated by surgical castration with (Fig. 6D) or without (Fig. 6C) testosterone (T, 30 mg/kg·wk) replacement according to a previously described protocol (12). Figure 7A reports the plasmatic concentration of T in the rats along with the weights of some androgen-target tissues such as seminal vesicles (Fig. 7B) and prostate (Fig. 7C). Castration dramatically reduced circulating T and weights of accessory glands (seminal vesicles and prostate), whereas T substitution fully restored them. SNP-induced relaxation was significantly higher in castrated rats (Fig. 6C) than in control rats ($P < 0.0001$) and was only slightly, but significantly ($P < 0.05$), increased by vardenafil (100 nM). Conversely, the SNP effect was not different in T-replaced castrated rats from that of untreated rats and was strongly increased by vardenafil (100 nM, Fig. 6D, $P < 0.0001$). Hormonal manipulation did not change the SNP IC_{50} (shared $IC_{50} = 2.34 \pm 1.7$ μM). We therefore hypothesized an androgen regulation of the vardenafil target, *i.e.* PDE5. Figure 7D shows results of androgen regulation of PDE5 gene expression in the rat bladder. Surgical castration induced a significant decrease ($P < 0.05$) of PDE5 mRNA as measured by real-time RT-PCR, which was rescued by T administration (Fig. 7D).

To elucidate whether PDE5 inhibition also has *in vivo* effects on detrusor overactivity, we next used the BOO rat model, in which a partial ligature of the urethra was performed under anesthesia, resulting in pronounced bladder hypertrophy and bladder NVCs. Five weeks after BOO surgery, the bladder weight was significantly increased in BOO rats, approximately eightfold compared with non-BOO animals (110 ± 22 mg). However, 5-wk treatment with either vardenafil or tamsulosin did not influence bladder hypertrophy in the BOO rats. Bladder weight was 873 ± 45 mg in the BOO placebo group and 862 ± 50 mg, 814 ± 85 mg, and 874 ± 66 mg in the 3 mg/kg·d vardenafil, the 10 mg/kg·d vardenafil, and the 10 μg/kg·d tamsulosin treatment groups, respectively (Fig. 8A). The bladder weight in the 10 mg/kg·d vardenafil-treated animals tended to decrease; however, this did not reach significance. After 5 wk of chronic treatment, a conscious cystometry was performed in the BOO rats (Fig. 8B). Vardenafil caused a 29% and 47% dose-dependent reduction of NVCs in the 3 and 10 mg/kg·d groups, respectively, which was significant at the highest vardenafil dose ($P < 0.05$). Figure 8B shows original tracings of a representative cystometrograph, whereas Figure 8C summarizes results on NVCs. Tamsulosin, a uroselective α (1A)-adrenergic receptor antagonist, which served as positive control, significantly ($P < 0.05$) reduced NVCs by 51% in comparison with the placebo group (Fig. 8C).

Discussion

This study demonstrates that PDE5 is present and biologically active in the human and rat bladder. We also show for the first time that inhibiting this enzyme with vardenafil, a selective PDE5 inhibitor, increases nitric oxide signaling, potentiates bladder relaxation, inhibits bladder smooth muscle proliferation, and decreases NVCs in BOO rats. That PDE5

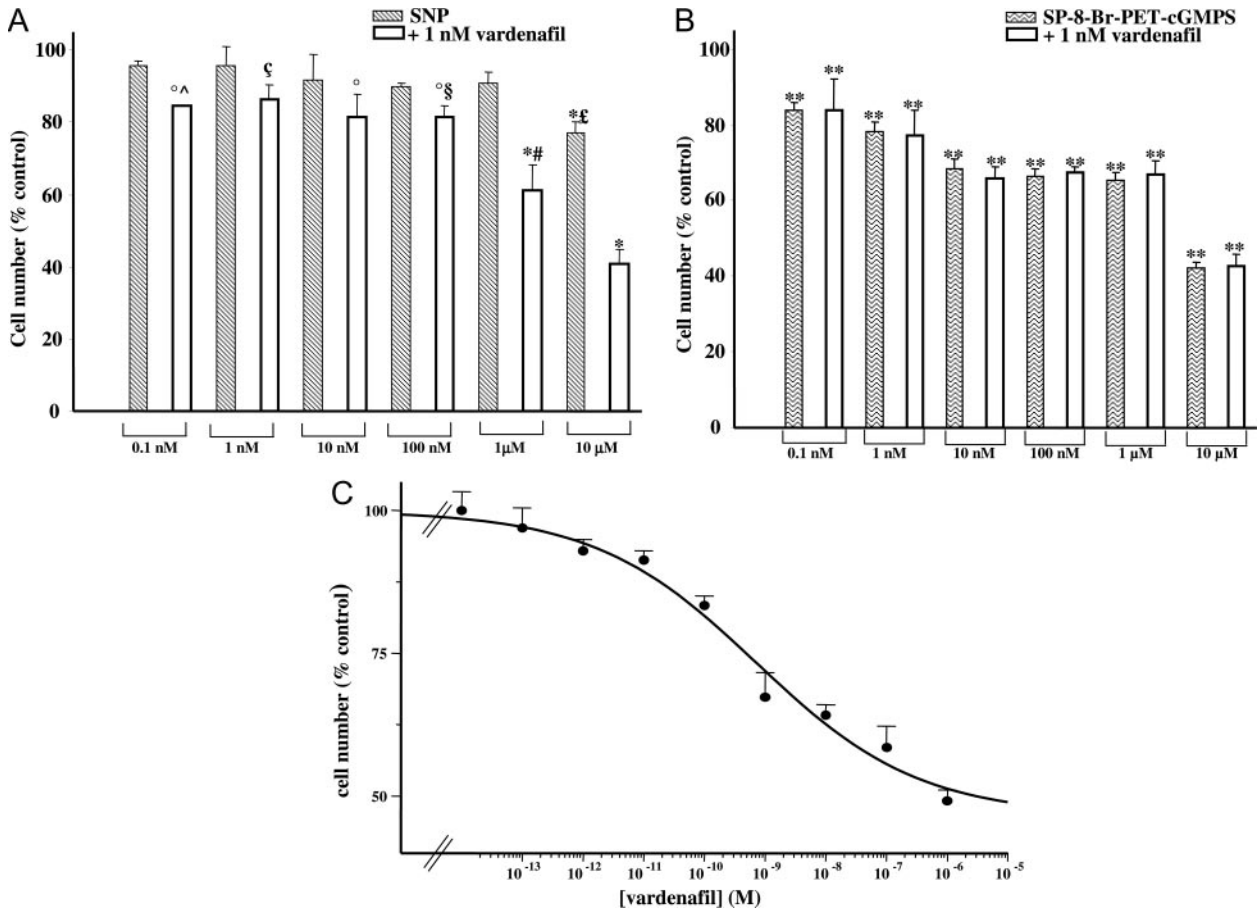

FIG. 5. Effect of nitric oxide signaling on proliferation of hBC. A, Effect of 48-h exposure to increasing concentrations of SNP (0.1 nM–10 μM) with or without vardenafil (1 nM) on hBC growth. *, $P < 0.005$ *vs.* C; °, $P < 0.05$ *vs.* C; ^, $P < 0.005$ *vs.* SNP 0.1 nM;ç, $P < 0.05$ *vs.* C; §, $P < 0.05$ *vs.* SNP 100 nM; #, $P < 0.05$ *vs.* SNP 1 μM;£, $P < 0.01$ *vs.* SNP 10 μM. B, Effect of 48-h exposure to increasing concentrations of SP-8-Br-PET-cGMPS (0.1 nM–10 μM) with or without vardenafil (1 nM) on hBC growth. **, $P < 0.0001$ *vs.* C. C, Effect of 48-h exposure to increasing concentrations of vardenafil (0.01 pM–1 μM) with SNP (1 μM) on hBC growth. Results are expressed as the percent variation (mean ± SEM) over the relative control value and are derived from four different experiments obtained from two distinct hBC cell preparations.

expression and activity are testosterone-dependent in the rat bladder are also novel results.

Nitric oxide synthase activity has been identified in the urothelium, smooth muscle, blood vessels, and nerves of the bladder, being higher in the outlet region (23, 24). However, it is generally assumed (see Refs. 1 and 2 for reviews) that the nitric oxide–cGMP pathway plays a minor, if any, role in regulating the bladder micturition cycle based on the only marginal SNP, SIN-1, and nitric oxide-induced bladder relaxation in several animal species (25–28). Nonetheless, a physiological role for nitric oxide was envisaged in inducing relaxation during the filling phase (29, 30). In addition, nitric oxide signaling was supposed to restrain bladder smooth muscle cell proliferation (31). In agreement with these observations, our results show that nitric oxide signaling only marginally affects bladder smooth muscle contraction and proliferation. However, a PDE-resistant cGMP analog, SP-8-Br-PET-cGMPS, substantially relaxes carbachol-precontracted rat bladder strips and inhibits proliferation of human bladder smooth muscle cells, suggesting that a cGMP-specific PDE activity may limit nitric oxide action. In this study, we characterize PDE activity in the human bladder by using a subset of inhibitors for cGMP-specific PDEs, including those selective for PDE5. We found that selective PDE5i, vardenafil, sildenafil, and tadalafil, reduced the majority (70%), but not all cGMP-metabolizing activity in human bladder homogenates. In contrast, a total inhibition of cGMP conversion into metabolites was observed with other less

TABLE 2. Time course of SNP-induced growth inhibition in hBC

Time (h)	Control	SNP
6	29,750 ± 750	30,000 ± 1,000 (100.84 ± 3.36)
12	29,000 ± 1,000	28,250 ± 2,250 (97.41 ± 7.76)
24	30,000 ± 1,000	31,750 ± 250 (105.83 ± 0.83)
48	30,500 ± 500	26,250 ± 250 (86.07 ± 0.82)[a]

Human bladder cells were treated with vehicle (control) or with the nitric oxide donor, SNP (1 μM), for the indicated times. *Numbers* indicate the mean cell number ± SEM in the different conditions. *Numbers in parentheses* indicate percentage variation over control. A significant inhibition of cell growth was obtained after 48-h exposure to the nitric oxide donor, SNP (1 μM).

[a] $P < 0.01$ *vs.* its relative control.

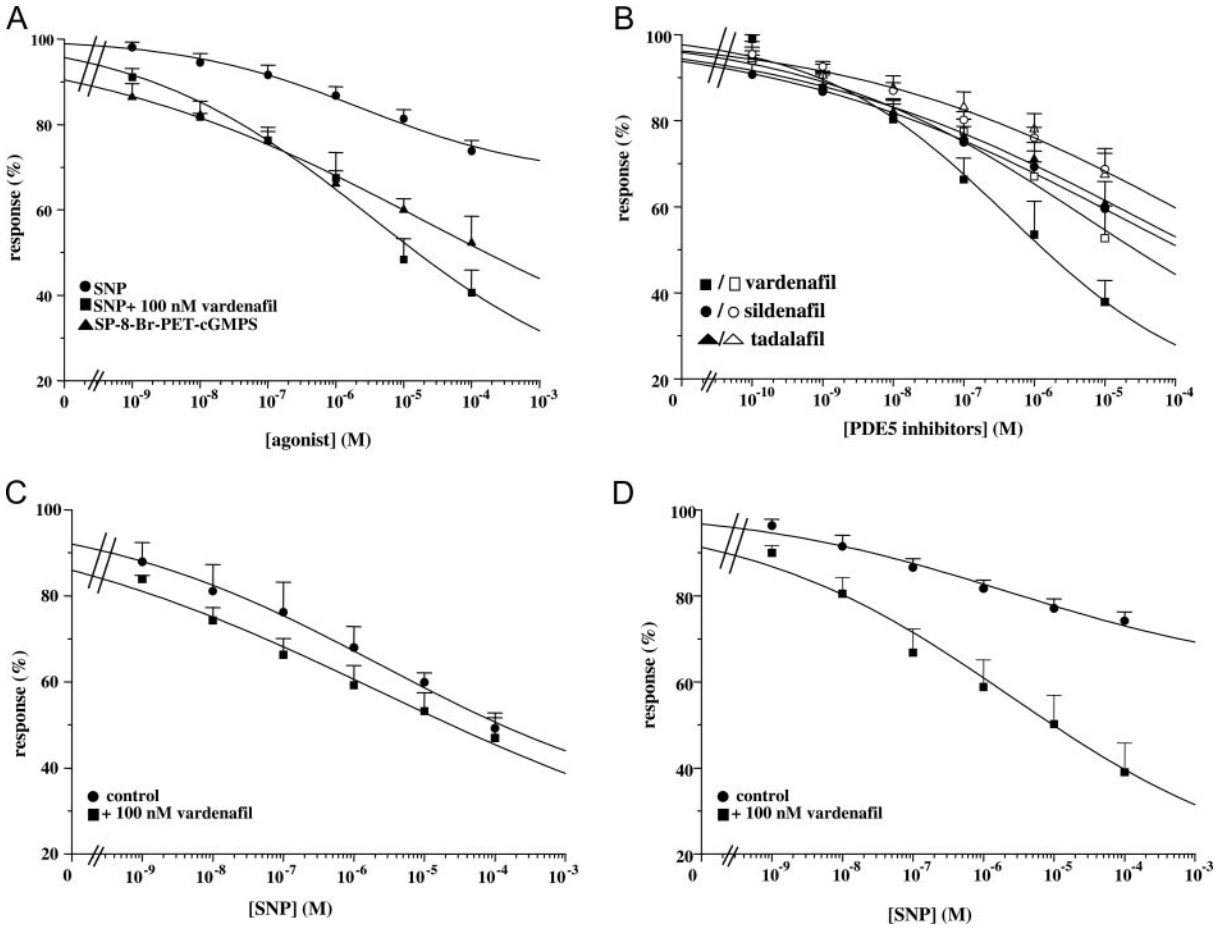

FIG. 6. Modulation by PDE5 of nitric oxide/cGMP-induced relaxation in rat bladder. A, C, D, Effect of inhibition of cGMP degradation (vardenafil, 100 nM, closed boxes) on the relaxant response to increasing concentrations of SNP in (A) control, (C) castrated, or (D) T-replaced castrated rats. A, The effect of the PDE-resistant cGMP analog SP-8-Br-PET-cGMPS (closed triangles) on rat bladder is also shown. B, Effect of different PDE5 inhibitors on rat bladder strips in the absence (open symbols) or in the presence (closed symbols) of a submaximal concentration of the nitric oxide donor SNP (0.3 μM). *Ordinate*: contractile tone induced by carbachol (10 μM). Maximal response to carbachol, before the addition of agonists, was taken as 100%. The effect of the relaxant agents was evaluated as a percentage of this response. Absolute values (in milligrams) of bladder contractions induced by carbachol (10 μM) are (A) 1021.2 ± 71.1 without vardenafil and 911.7 ± 104.8 with vardenafil, (C) 961.4 ± 96.4 without vardenafil and 768.7 ± 49.1 with vardenafil, (D) 1261.5 ± 110.9 without vardenafil and 1002.4 ± 134.3 with vardenafil. B, The absolute values (in milligrams) of bladder contractions induced by carbachol (10 μM) are for vardenafil without SNP = 1235.0 ± 159.2 and with SNP = 960.0 ± 214.7; for sildenafil without SNP = 1067.7 ± 113.3 and with SNP = 1111.2 ± 179.3; for tadalafil without SNP = 1508.2 ± 202.4 and with SNP = 1080.2 ± 207.9. None of these values were statistically different. *Abscissa*: concentration of the indicated agents. Each *point* represents mean ± SEM of at least five experiments. The relative EC_{50} values are reported in the text.

selective PDE5i, dipyridamole and zaprinast, which also interact at similar concentrations also with other cGMP PDEs such as PDE6, PDE9, and PDE10 (21, 22, 32), indicating that these PDEs are also present and biologically active in the human bladder. However, their characterization was beyond the aim of the present study. Interestingly, the rank order of potency of cGMP-selective PDE5i in the human bladder is very similar to that previously found in the human corpora cavernosa (13, 19) with vardenafil being the most potent compound tested. Accordingly, vardenafil was also the most potent PDE5i in inducing rat bladder strips relaxation. Altogether, these results confirm a previous observation demonstrating a sildenafil-sensitive cGMP-metabolizing activity in the rat bladder (28) and suggest the presence of a PDE5-like cGMP-metabolizing activity in the human bladder. The role of PDE5 in the bladder was further supported by gene expression and tissue immunolocalization studies. As already reported (10, 11), quantitative RT-PCR studies confirmed that PDE5 is expressed in the human bladder at the same level as other portions of the male genital tract but lower than in corpora cavernosa, whereas in the rat bladder, PDE5 is even higher than in penile extracts. According to previous studies in other human tissues (11, 19, 33, 34), PDE5 is selectively expressed in the endothelial and smooth muscle cells of the bladder vessel and rather abundantly in the muscle fibers.

Blocking PDE5 with vardenafil strongly increased nitric oxide signaling in bladders to an extent similar to that obtained with the PDE-resistant cGMP analog as derived from experiments in isolated smooth muscle cells or bladder strips. This indicates a relevant role played by PDE5 in limiting nitric oxide–cGMP signaling in the bladder, providing an explanation about the low efficacy of nitric oxide donors observed in previous studies (see Refs. 1 and 2 for reviews).

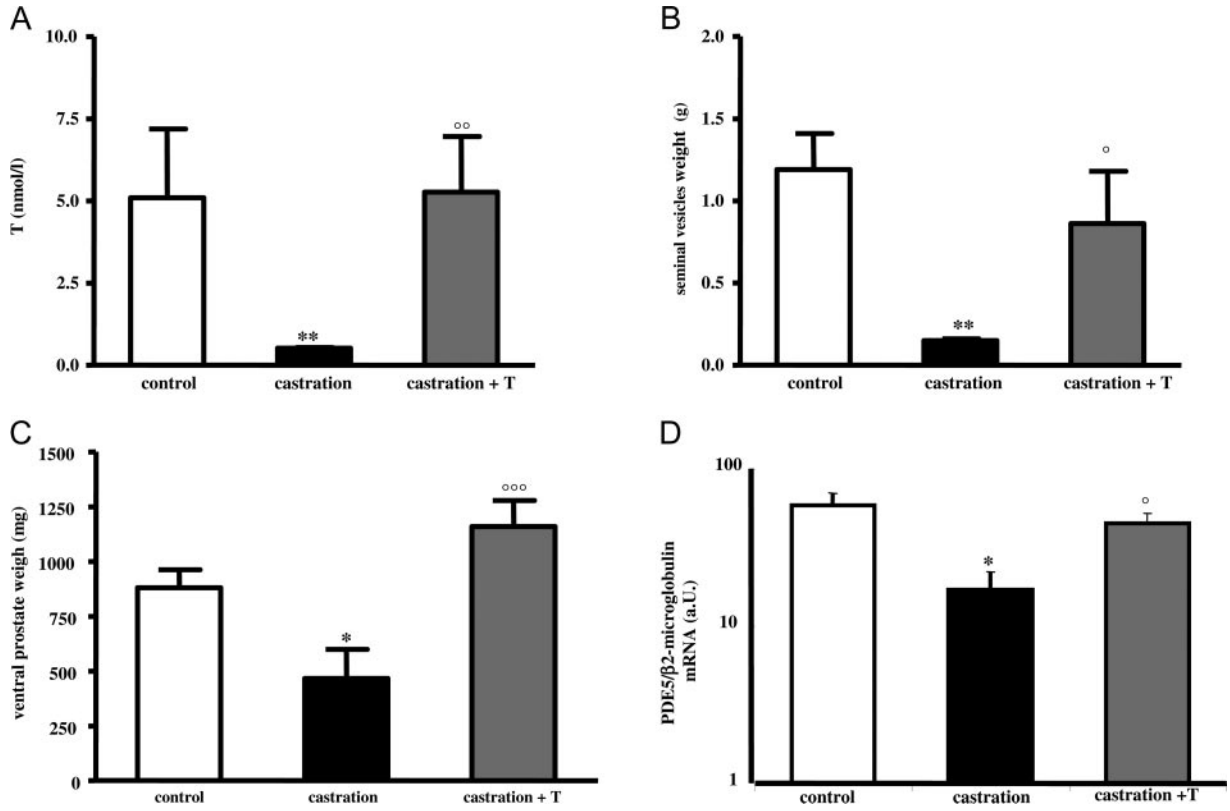

FIG. 7. Effects of castration and T replacement on T levels, male accessory gland weight, and on PDE5 expression in rat bladder. *White columns* represent results in intact animals (control); *black columns* in castrated animals; *gray columns* in T-replaced castrated animals. *Bars* indicate SEM *, $P < 0.05$; **, $P < 0.0001$ *vs.* control; °, $P < 0.05$; °°, $P < 0.001$; °°°, $P < 0.0001$ *vs.* castration. A–C, Male accessory glands (B, seminal vesicles; C, prostate) and blood for T measurement (A) were collected 2 wk after surgical castration and 1 wk after the last injection of T enanthate (30 mg/kg·wk). Data are derived from at least seven experiments for each group. D, Quantitative detection of mRNA for PDE5 in bladder tissues from experimental rats by real-time RT-PCR. Number of experimental observations (n) were: control n = 13, castration n = 6, T replacement n = 8. Data are expressed in arbitrary units (a.U.) calculated according to the comparative Ct method and using the β_2-microglobulin as reference gene for normalization.

The PDE5i-induced cGMP increase might directly relax the smooth muscle compartment or indirectly act through crossinhibition of cGMP-dependent cAMP PDE (PDE3) with the final result of increasing cAMP (35). In fact, the cAMP pathway is generally viewed as more potent in relaxing the bladder than the cGMP pathways (1, 2). Interactions between the smooth muscle-generated cGMP and other bladder compartments (such as the urothelium–suburothelium, interstitial cells, or nerve afferents) should also be considered in view of their relevant roles in controlling the micturition cycle (1, 2). However, it is noteworthy that PDE5 in the human bladder (apart from endothelium of the blood vessels) is expressed only in the smooth muscle cells, whereas other compartments such as urothelium–suburothelium are definitively negative.

Because we had previously found that PDE5 expression and activity in corpora cavernosa and vas deferens is androgen-regulated (11, 15, 19, 20) and the bladder expresses androgen receptors (14, 36), we hypothesized a PDE5 androgen-dependency also in the bladder. We essentially found that bladder strips from castrated rats were more sensitive to SNP-induced relaxation and less responsive to vardenafil, suggesting that androgen deprivation might down-regulate PDE5 activity. Accordingly, PDE5 gene expression was significantly reduced by surgical castration. T replacement fully restored PDE5 expression, along with responsiveness to vardenafil, and decreased SNP-induced relaxation.

Overall, the aforementioned results indicate that PDE5 might play a physiological role in controlling nitric oxide–cGMP-regulated bladder smooth muscle relaxation and growth inhibition. The PDE5 inhibitors might therefore represent an interesting possibility for conditions such as BPH-induced bladder overactivity characterized by both bladder overgrowth and unregulated muscular contractility. We therefore tested the effect of continuous exposure to vardenafil in an experimental model of BOO obtained by partial urethra ligation, which induces bladder NCVs and hypertrophy. Vardenafil was selected on the basis of our *in vitro* studies. Its effects were compared with those of the uroselective α (1A) adrenergic receptor antagonist tamsulosin. BOO-induced NVCs have been considered a correlate of the BPH-related detrusor overactivity, and this model has previously been used for testing the efficacy of α-blockers on LUTS (37). In the BOO model, we found that vardenafil dose-dependently decreases NCVs to an extent similar to tamsulosin but only marginally affects bladder overgrowth.

In conclusion, our results suggest that PDE5 positively

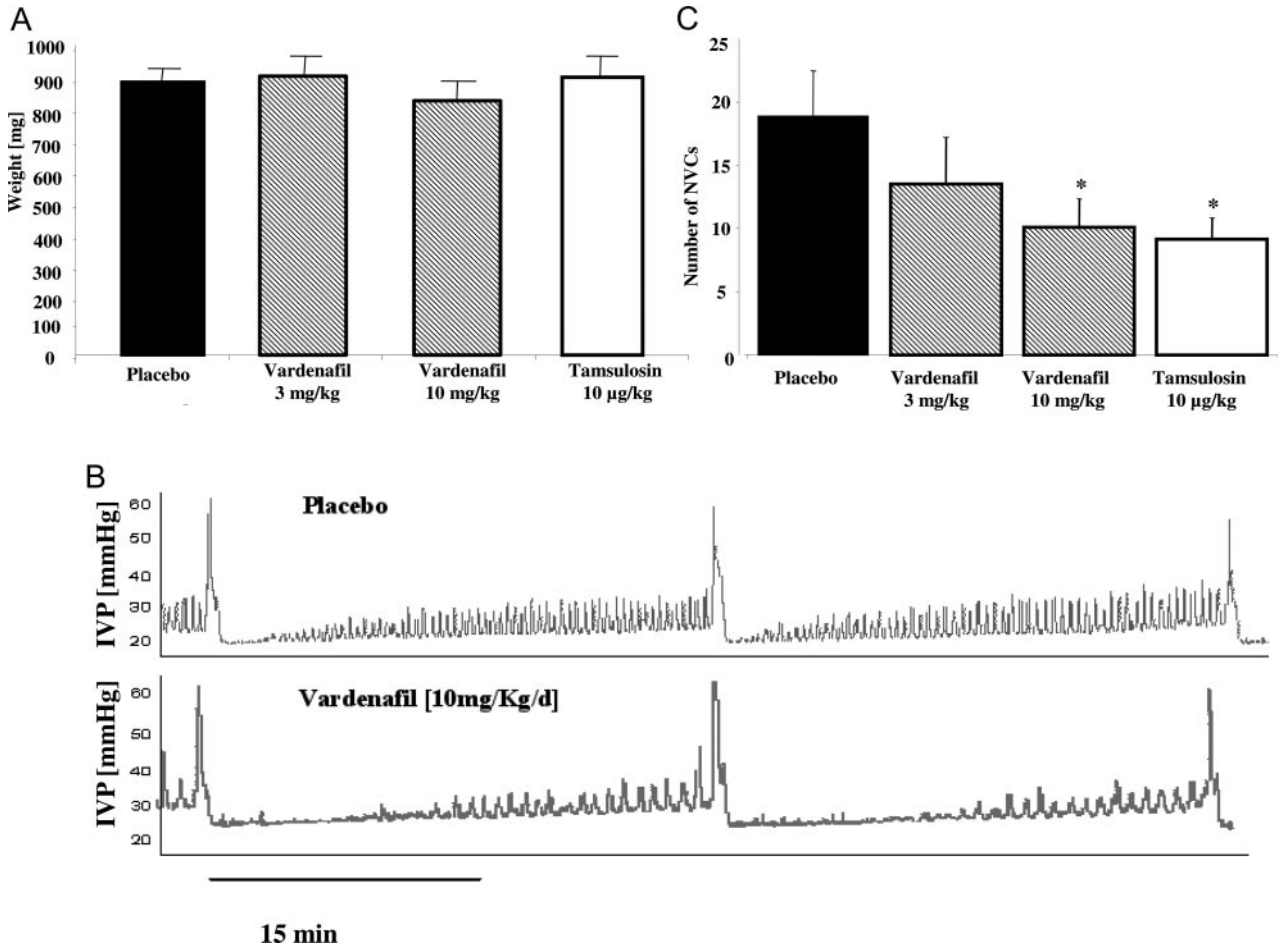

FIG. 8. Effect of vardenafil treatment on BOO rat model. A, Bladder weight in milligrams of BOO rats treated for 5 wk with placebo, 3 mg/kg·d and 10 mg/kg·d vardenafil, and 10 μg/kg·d tamsulosin. B, Tracing of a cystometry showing IntraVesical Pressure (IVP in mm Hg) over time (min) in BOO rats after 5 wk of treatment with placebo (*upper panel*) and 10 mg/kg·d vardenafil (*lower panel*). C, Number of NVCs in BOO rats treated for 5 wk with placebo, 3 mg/kg·d and 10 mg/kg·d vardenafil, and 10 μg/kg·d tamsulosin. Data are mean ± SEM; n = 10–15 animals/treatment group; *, $P < 0.05$ *vs.* placebo.

stimulates bladder *in vitro* and *in vivo* activity and that its inhibition might ameliorate overactivity, amplifying nitric oxide signaling. Although *in vitro* studies indicate that vardenafil might also restrain smooth muscle cell proliferation, experiments *in vivo* do not corroborate this notion, at least in BOO conditions, characterized by robust muscle hypertrophy.

BPH is an androgen-dependent disease. In fact, androgen ablation (through GnRH agonists, androgen receptor antagonists, or dihydrotesterone inhibitor formation) is an effective strategy in reducing prostate size and LUTS (38). The positive effects of androgen ablation on LUTS are generally attributed to the reduction in prostate size and to decreased urethral obstruction. However, androgen ablation might also ameliorate at some stage the neural circuits involved in controlling urine storage (see Ref. 39 for review). Alternatively, it is possible that the beneficial effect of androgen ablation on LUTS are mediated by reduced expression of PDE5, which is androgen-dependent in the rat bladder, and therefore by an enhancement of nitric oxide-induced relaxation during the filling phase. The androgen dependency of PDE5 could, at least partially, explain sex differences in bladder function in genetic models of neuronal nitric oxide synthase deficiency. In fact, voiding studies reveal increased micturition frequency and bladder weight in male neuronal nitric oxide synthase knockout mice (40), whereas the same is not apparent in female neuronal nitric oxide synthase-deficient mice (41). This may indicate that male mice have more difficulty in compensating for lower nitric oxide formation (through other nitric oxide synthases) than females, most probably because cGMP degradation through PDE5 is more active in males than in females (because of its androgen dependency). Further functional studies using different sex steroid replacement such as estrogens and not aromatizable androgens are needed to clarify these points. In addition, whether the same androgen dependency of PDE5 reported here in the rat bladder operates also in the human bladder should be investigated.

The present results are in line with other reports showing a positive effect of PDE5 inhibition in men with ED and BPH in uncontrolled (4–6) and placebo-controlled (7–9) clinical trials. The possibility that vardenafil, as well as other PDE5i, ameliorates the dynamic component of irritative BPH-induced LUTS deserves further clinical investigation.

Acknowledgments

We thank Prof. Fabrizio Ledda (Department of Pharmacology, University of Florence, Florence, Italy) for helpful suggestions during the course of the study; and "Centro per i servizi di Stabulazione degli Animali di Laboratorio" (Ce.S.A.L.), University of Florence, Florence, Italy, for technical assistance in animal treatment.

Received August 9, 2006. Accepted November 25, 2006.

Address all correspondence and requests for reprints to: Mario Maggi, Andrology Unit, Department of Clinical Physiopathology, University of Florence, V.le G. Pieraccini, 6, 50139 Florence, Italy. E-mail: m.maggi@dfc.unifi.it.

Disclosure Statement: Sandra Filippi, Annamaria Morelli, Benedetta Fibbi, Rosa Mancina, Mirca Marini, Mauro Gacci, Linda Vignozzi, Gabriella Barbara Vannelli, Marco Carini, and Gianni Forti have nothing to declare. Mario Maggi is a scientific consultant for Bayer Health Care AG. Peter Sandner is an employee of Bayer Health Care AG.

References

1. **Andersson KE, Arner A** 2004 Urinary bladder contraction and relaxation: physiology and pathophysiology. Physiol Rev 84:935–986
2. **Andersson KE, Wein AJ** 2004 Pharmacology of the lower urinary tract: basis for current and future treatments of urinary incontinence. Pharmacol Rev 56:581–631
3. **Gacci M, Bartoletti R, Figlioli S, Sarti E, Eisner B, Boddi V, Rizzo M** 2003 Urinary symptoms, quality of life and sexual function in patients with benign prostatic hypertrophy before and after prostatectomy: a prospective study. BJU Int 91:196–200
4. **Sairam K, Kulinskaya E, McNicholas TA, Boustead GB, Hanbury DC** 2002 Sildenafil influences lower urinary tract symptoms. BJU Int 90:836–839
5. **Mulhall JP, Guhring P, Parker M, Hopps C** 2006 Assessment of the impact of sildenafil citrate on lower urinary tract symptoms in men with erectile dysfunction. J Sex Med 3:662–667
6. **Chang SH** 2006 Influence of sildenafil on lower urinary tract symptoms [Abstract]. J Urol 175(suppl):299
7. **Mc Vary KT** 2006 Sildenafil improves erectile function and concomitant lower urinary tract symptoms in men. J Urol 175(Suppl):298 (Abstract)
8. **Mc Vary KT** 2006 Sildenafil improves erectile function and urinary symptoms in men with erectile dysfunction and concomitant LUTS. J Urol 175(Suppl):527 (Abstract)
9. **Roehrborn CG** 2006 The efficacy and safety of tadalafil administered once day for lower urinary tract symptoms (LUTS) in men with benign prostatic hyperplasia. J Urol 175(Suppl):527 (Abstract)
10. **Stacey P, Rulten S, Dapling A, Phillips SC** 1998 Molecular cloning and expression of human cGMP-binding cGMP-specific phosphodiesterase (PDE5). Biochem Biophys Res Commun 247:249–254
11. **Morelli A, Filippi S, Mancina R, Luconi M, Vignozzi L, Marini M, Orlando C, Vannelli GB, Aversa A, Natali A, Forti G, Giorgi M, Jannini EA, Ledda F, Maggi M** 2004 Androgens regulate phosphodiesterase type 5 expression and functional activity in corpora cavernosa. Endocrinology 145:2253–2263
12. **Crescioli C, Ferruzzi P, Caporali A, Mancina R, Comerci A, Muratori M, Scaltriti M, Vannelli GB, Smiroldo S, Mariani R, Villari D, Bettuzzi S, Serio M, Adorini L, Maggi M** 2003 Inhibition of spontaneous and androgen-induced prostate growth by a nonhypercalcemic calcitriol analog. Endocrinology 144: 3046–3057
13. **Morelli A, Vignozzi L, Filippi S, Vannelli GB, Ambrosini S, Mancina R, Crescioli C, Donati S, Fibbi B, Colli E, Adorini L, Maggi M** 2006 BXL-628, a vitamin D receptor agonist effective in benign prostatic hyperplasia treatment, prevents RhoA activation and inhibits RhoA/Rho kinase signaling in rat and human bladder. Prostate 67:234–247
14. **Crescioli C, Morelli A, Adorini L, Ferruzzi P, Luconi M, Vannelli GB, Marini M, Gelmini S, Fibbi B, Donati S, Villari D, Forti G, Colli E, Andersson KE, Maggi M** 2005 Human bladder as a novel target for vitamin D receptor ligands. J Clin Endocrinol Metab 90:962–972
15. **Zhang XH, Filippi S, Morelli A, Vignozzi L, Luconi M, Donati S, Forti G, Maggi M** 2005 Testosterone regulates PDE5 expression and in vivo responsiveness to tadalafil in rat corpus cavernosum. Eur Urol 47:409–416
16. **Bradford MM** 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254
17. **D'Amati G, di Gioia CR, Bologna M, Giordano D, Giorgi M, Dolci S, Jannini EA** 2002 Type 5 phosphodiesterase expression in the human vagina. Urology 60:191–195
18. **De Lean A, Munson PJ, Rodbard D** 1978 Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose–response curves. Am J Physiol 235:E97–E102
19. **Mancina R, Filippi S, Marini M, Morelli A, Vignozzi L, Salonia A, Montorsi F, Mondaini N, Vannelli GB, Donati S, Lotti F, Forti G, Maggi M** 2005 Expression and functional activity of phosphodiesterase type 5 in human and rabbit vas deferens. Mol Hum Reprod 11:107–115
20. **Morelli A, Filippi S, Vignozzi L, Mancina R, Maggi M** 2006 Physiology of erectile function: an update on intracellular molecular processes. EAU-EBU Update Series 4:96–108
21. **Soderling SH, Bayuga SJ, Beavo JA** 1998 Cloning and characterization of a cAMP-specific cyclic nucleotide phosphodiesterase. Proc Natl Acad Sci U S A 95:8991–8996
22. **Soderling SH, Bayuga SJ, Beavo J** 1998 Identification and characterization of a novel family of cyclic nucleotide phosphodiesterases. J Biol Chem 273:15553–15558
23. **Mumtaz FH, Khan MA, Thompson CS, Morgan RJ, Mikhailidis DP** 2000 Nitric oxide in the lower urinary tract: physiological and pathological implications. BJU Int 85:567–578
24. **Hedlund P** 2005 Nitric oxide/cGMP-mediated effects in the outflow region of the lower urinary tract—is there a basis for pharmacological targeting of cGMP? World J Urol 23:362–367
25. **Persson K, Andersson KE** 1992 Nitric oxide and relaxation of pig lower urinary tract. Br J Pharmacol 106:416–422
26. **Morita T, Tsujii T, Dokita S** 1992 Regional difference in functional roles of cAMP and cGMP in lower urinary tract smooth muscle contractility. Urol Int 49:191–195
27. **Persson K, Alm P, Johansson K, Larsson B, Andersson KE** 1993 Nitric oxide synthase in pig lower urinary tract: immunohistochemistry, NADPH diaphorase histochemistry and functional effects. Br J Pharmacol 110:521–530
28. **Qiu Y, Kraft P, Craig EC, Liu X, Haynes-Johnson D** 2001 Identification and functional study of phosphodiesterases in rat urinary bladder. Urol Res 29: 388–392
29. **James MJ, Birmingham AT, Hill SJ** 1993 Partial mediation by nitric oxide of the relaxation of human isolated detrusor strips in response to electrical field stimulation. Br J Clin Pharmacol 35:366–372
30. **Theobald RJ Jr** 1996 The effect of N^G-monomethhyl-L-arginine on bladder function. Eur J Pharmacol 311:73–78
31. **Johansson R, Pandita RK, Poljakovic M, Garcia-Pascual A, De Vente J, Persson K** 2002 Activity and expression of nitric oxide synthase in the hypertrophied rat bladder and the effect of nitric oxide on bladder smooth muscle growth. J Urol 168:2689–2694
32. **Fujishige K, Kotera J, Michibata H, Yuasa K, Takebayashi S, Okumura K, Omori K** 1999 Cloning and characterization of a novel human phosphodiesterase that hydrolyzes both cAMP and cGMP (PDE10A). J Biol Chem 274: 18438–18445
33. **Black SM, Sanchez LS, Mata-Greenwood E, Bekker JM, Steinhorn RH, Fineman JR** 2001 sGC and PDE5 are elevated in lambs with increased pulmonary blood flow and pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 281:L1051–L1057
34. **Dolci S, Belmonte A, Santone R, Giorgi M, Pellegrini M, Carosa E, Piccione E, Lenzi A, Jannini EA** 2006 Subcellular localization and regulation of type-1C and type-5 phosphodiesterases. Biochem Biophys Res Commun 341:837–846
35. **Uckert S, Hedlund P, Andersson KE, Truss MC, Jonas U, Stief CG** 2006 50:1194–1207 Update on phosphodiesterase (PDE) isoenzymes as pharmacologic targets in urology: present and future. Eur Urol 50:1194–1207
36. **Rosenzweig BA, Bolina PS, Birch L, Moran C, Marcovici I, Prins GS** 1995 Location and concentration of estrogen, progesterone, and androgen receptors in the bladder and urethra of the rabbit. Neurourol Urodyn 14:87–96
37. **Gu B, Reiter JP, Schwinn DA, Smith MP, Korstanje C, Thor KB, Dolber PC** 2004 Effects of alpha 1-adrenergic receptor subtype selective antagonists on lower urinary tract function in rats with bladder outlet obstruction. J Urol 172:758–762
38. **Maggi M, Crescioli C, Morelli A, Colli E, Adorini L** 2006 Pre-clinical evidence and clinical translation of benign prostatic hyperplasia treatment by the vitamin D receptor agonist BXL-628 (Elocalcitol). J Endocrinol Invest 29:665–674
39. **Keast JR** 1999 The autonomic nerve supply of male sex organs—an important target of circulating androgens. Behav Brain Res 105:81–92
40. **Burnett AL, Calvin DC, Chamness SL, Liu JX, Nelson RJ, Klein SL, Dawson VL, Dawson TM, Snyder SH** 1997 Urinary bladder-urethral sphincter dysfunction in mice with targeted disruption of neuronal nitric oxide synthase models idiopathic voiding disorders in humans. Nat Med 3:571–574
41. **Sutherland RS, Kogan BA, Piechota HJ, Bredt DS** 1997 Vesicourethral function in mice with genetic disruption of neuronal nitric oxide synthase. J Urol 157:1109–1116