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Platinum Priority – Review – Benign Prostatic Hyperplasia

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The Mechanism of Action of Phosphodiesterase Type 5 Inhibitors in the Treatment of Lower Urinary Tract Symptoms Related to Benign Prostatic Hyperplasia

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Abstract

Context: Clinical trials of phosphodiesterase type 5 inhibitors (PDE5-Is) have consistently demonstrated a significant reduction in lower urinary tract symptoms (LUTS) and small urinary flow rate changes in men with benign prostatic hyperplasia (BPH).
Objective: This review presents the proposed mechanisms of action of PDE5-Is in the treatment of BPH-LUTS focusing on the localization of PDE5 isoenzymes in the pelvic structures; smooth muscle relaxation in the bladder, prostate, and supporting vasculature; increased blood perfusion of the bladder and prostate; and modulation of sensory impulses from these organs.
Evidence acquisition: Literature describing in vitro, preclinical, or clinical studies of pathologic processes contributing to LUTS or effects of PDE5 inhibition on the lower urinary tract (LUT) was selected for review.
Evidence synthesis: We objectively assessed and summarized the published data focusing on articles published within the past 10 yr. Articles before the time cut-off were included if historically relevant.
Conclusions: The PDE5 isoenzymes are highly expressed in the LUT including the bladder, prostate, and their supporting vasculature. In vitro assays have demonstrated PDE5-Is by regulating cyclic guanosine monophosphate (cGMP) degradation and enhancing the nitric oxide/cGMP signaling pathway to relax human smooth muscle strips from the prostate, bladder, and LUT arteries. In animals characterized by ischemia/hypoxia of the genitourinary tract, treatment with PDE5-Is increases bladder and prostate tissue oxygenation. PDE5-Is have been shown to reduce nonvoiding contractions and bladder afferent nerve firing in decerebrate spinal cord–injured rats, and to reduce mechanosensitive afferent activities of both Aδ- and C-fibers in an irritated or overextended bladder model.

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1. Introduction

The normal micturition cycle in the male is a complex process involving the bladder, prostate, and urethra as well as the pelvic neuronal and vascular networks innervating and perfusing these organs (Fig. 1). The tone, contractions, and relaxation of the smooth detrusor muscle in the bladder and bladder neck, as well as the smooth and striated sphincters in the urethra, are mediated by a multifaceted central and peripheral autonomic and somatic neural control system coordinated in the spinal cord and brain.

The smooth muscle tone in the lower urinary tract (LUT) is controlled by various adrenergic, cholinergic, and nonadrenergic noncholinergic neurotransmitters released from nerve terminals and endogenous factors from vascular endothelial sources. The nitric oxide (NO)/cyclic guanosine monophosphate (cGMP)–mediated pathway and related key enzymes including phosphodiesterase type 5 (PDE5) have been shown to play a central role in relaxant responses of LUT tissue.

During the storage of urine, the parasympathetic innervation of the detrusor is inhibited and the urethral sphincter is contracted preventing involuntary bladder emptying. This occurs because of (1) the activation of the sympathetic innervation conveyed by the hypogastric nerves to the bladder neck and the urethra, and (2) the recruitment of pudendal motor neurons to the external urethral sphincter. This guarding reflex is activated by bladder afferents conveyed by the pelvic nerves with distension of the bladder producing low-level bladder afferent firing. The bladder afferents consist of myelinated (Aδ) and unmyelinated (C) axons. The Aδ-fibers respond to passive distension and active contraction and thus convey information about bladder filling. The C-fibers are considered insensitive to bladder filling under physiologic conditions and accordingly termed "silent" C-fibers. However, evidence suggests that C-fibers may become mechanosensitive under pathologic conditions, providing nociceptive afferents to overdistension, inflammation, or irritation. The urothelium has specialized sensory and signaling properties to engage in chemical communication with nerves in the bladder wall. Urothelium can regulate the activity of adjacent nerves and thereby trigger local vascular changes and/or reflex bladder contractions. During the elimination of urine, intense bladder-afferent firing activates reflex pathways that pass through the pons. This stimulates the parasympathetic outflow to the bladder and to the urethral smooth muscle and inhibits the sympathetic and pudendal outflow to the urethral outlet [1].

Detrusor overactivity, prostate obstruction, and altered anatomic structures in and around the LUT and its vascular supply are important factors for the development of lower urinary tract symptoms (LUTS). Benign prostatic hyperplasia (BPH) is a histologic diagnosis characterized by smooth muscle and epithelial cell proliferation in the prostate transition zone, leading to nonmalignant prostate enlargement [2]. Although prostate enlargement due to BPH has long been associated with LUTS, it is widely recognized that it is not the exclusive cause. Pathophysiologic risk factors for LUTS suggestive of BPH (BPH-LUTS) include pelvic reduction in nitric oxide synthase (NOS)/NO, atherosclerosis/pelvic ischemia, autonomic overactivity, altered androgen environment, and local inflammation [3,4].

Tadalafil for once-daily use, a long-acting PDE5 inhibitor (PDE5-I), represents the first new class of drug approved by the US Food and Drug Administration in the past 20 yr for men with BPH-LUTS and for men with coexisting erectile dysfunction (ED) and BPH-LUTS. Clinical studies showed that tadalafil improved symptoms of BPH, including both storage and voiding symptoms, without the sexual dysfunction side effects seen in other BPH-LUTS treatments [5–10]. However, peak urinary maximum flow rate (Q_{max}) per uroflowmetry, although improved for both tadalafil and placebo, was typically not statistically different. Because of the small Q_{max} changes but the consistent, significant, and clinically meaningful improvement in urinary symptoms, questions have arisen about the mechanism of action for a PDE5-I such as tadalafil in the treatment of BPH-LUTS, although it is generally recognized that there is poor correlation between symptoms and Q_{max} [11].

This review provides an updated and simplified evaluation of the potential mechanism of action (MOA) as it relates to PDE5 inhibition and the clinical improvement in

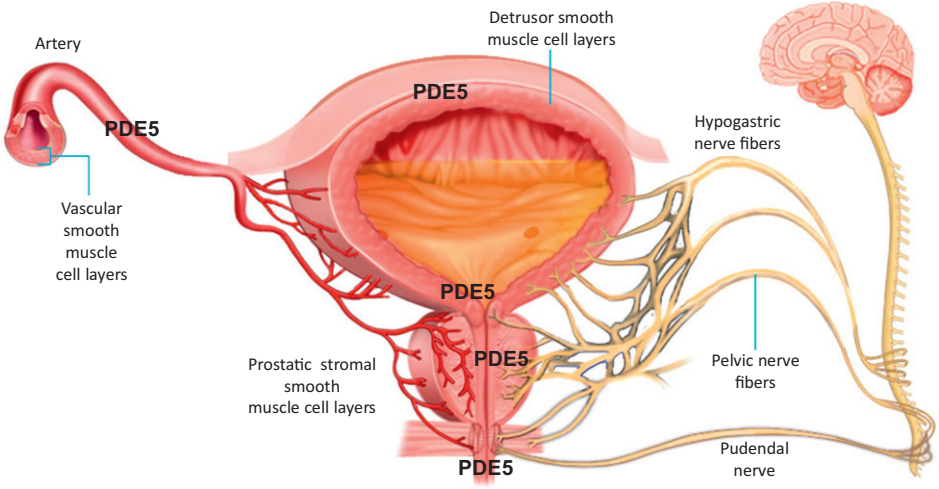

Fig. 1 – Phosphodiesterase type 5 (PDE5) isoenzymes in the lower urinary tract.

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BPH-LUTS focusing on the localization of PDE5 isoenzymes in the pelvic structures; smooth muscle relaxation in the bladder, prostate, and supporting vasculature; increased blood perfusion of the bladder and prostate; and modulation of sensory impulses from these organs.

2. Evidence acquisition

Literature was obtained via Medline searches and from the individual reviewer's files. Articles were selected that describe in vitro, preclinical, or clinical studies of pathologic processes contributing to LUTS or possible effects of PDE5 inhibition in the LUT. Only studies published in English were included. Relevant reference lists in the respective literature were also surveyed. When evaluating the effect of PDE5-Is on BPH symptom improvement in humans, only randomized placebo-controlled, double-blind clinical trials were included (level 1 evidence). Articles published within the past 10 yr were prioritized; however, older articles were included if they were of historical clinical significance.

3. Evidence synthesis

3.1. *Clinical studies assessing phosphodiesterase type inhibitors in men with benign prostatic hyperplasia-lower urinary tract symptoms*

Clinical trials of PDE5-Is have consistently shown reduction in storage and voiding symptoms as assessed by the International Prostate Symptom Score (IPSS) questionnaire (Table 1) [5,7,8,12–18]. In several large placebo-controlled studies with tadalafil, improvement in BPH symptoms was seen within 1–2 wk [5,7,8], and long-term efficacy was maintained during a 1-yr uncontrolled study [19]. The short- and long-term reduction in both storage and voiding symptoms may suggest a mechanism that involves multiple areas of the LUT. PDE5-Is with a shorter half-life, such as sildenafil and vardenafil or the modified released PDE5-I UK-369003, have also shown improvement in BPH symptoms in single randomized placebo-controlled studies; however, these results have not been reproduced, and the molecules have not been approved for use in men with BPH-LUTS [12–14].

Although the symptom improvements observed with PDE5-Is and α-blockers are similar, changes in Q_{max} with PDE5-Is have typically not been significantly different than placebo (Table 1). Interestingly, in the only large placebo-controlled study conducted in men with BPH-LUTS with tadalafil and tamsulosin, a small but significant Q_{max} change was reported with both tadalafil and tamsulosin compared with placebo [7]. The significant but modest changes in Q_{max} observed in α-blocker trials were attributed to relaxation of the bladder neck/prostatic smooth muscle cell layer [20]. In vitro studies have also found that PDE5-Is relax the bladder/prostatic smooth muscle cell layers [21–23], but why this effect does not consistently translate into significant Q_{max} changes is unclear. Whether or not these small changes in Q_{max} identified with both α-blockers

Table 1 – Mean changes from baseline to end point in total International Prostate Symptom Score (IPSS), IPSS subscores, and maximum flow rate in double-blind randomized, placebo-controlled clinical studies of phosphodiesterase type 5 inhibitors

Study	Duration	Treatment	*n*	Total IPSS[a]	IPSS storage subscore[a]	IPSS voiding subscore[a]	Q_{max}, ml/s
Tadalafil							
McVary et al. [6]	12 wk	Placebo	143	−1.7	−1.0	−0.7	0.9[a]
		Tadalafil 5 mg/20 mg	138	−3.8*	−2.2*	−1.7*	0.5[a]
Roehrborn et al. [9]	12 wk	Placebo	210	−2.3	−1.0	−1.3	1.2[a]
		Tadalafil 2.5 mg	208	−3.9*	−1.6	−2.2*	1.4[a]
		Tadalafil 5 mg	212	−4.9*	−1.9*	−2.9*	1.6[a]
		Tadalafil 10 mg	216	−5.2*	−2.0*	−3.1*	1.6[a]
		Tadalafil 20 mg	208	−5.2*	−2.1*	−3.1*	2.0[a]
Porst et al. [8]	12 wk	Placebo	164	−3.6	−1.3	−2.3	1.1[a]
		Tadalafil 5 mg	161	−5.6*	−2.3*	−3.3*	1.6[a]
Egerdie et al. [5]	12 wk	Placebo	200	−3.8	−1.6	−2.2	1.2[a]
		Tadalafil 2.5 mg	198	−4.6	−1.9	−2.7	1.7*,[a]
		Tadalafil 5 mg	208	−6.1*	−2.5*	−3.6*	1.6[a]
Oelke et al. [7]	12 wk	Placebo	172	−4.2	−1.6	−2.6	1.2 [a]
		Tadalafil 5 mg	171	−6.3*	−2.2	−4.1*	2.4*,[a]
		Tamsulosin 0.4 mg	165	−5.7*	−2.2	−3.5*	2.2*,[a]
Sildenafil							
McVary et al. [13]	12 wk	Placebo	162	−1.9	VNR[d]	VNR[d]	0.2[a]
		Sildenafil 50 or 100 mg	179	−6.3*	VNR*,[d]	VNR*,[d]	0.3[a]
Vardenafil							
Stief et al. [14]	8 wk	Placebo	110	−3.6	−1.6	−1.9	1.0[b]
		Vardenafil 10 mg[c]	104	−5.9*	−2.7*	−3.2*	1.6[b]

IPSS = International Prostate Symptom Score; Q_{max} = maximum flow rate; VNR = value not reported.
[a] Mean change from baseline to end point.
[b] Change calculated by subtracting results reported at 8 wk from baseline.
[c] Twice daily.
[d] Subscores were reported graphically without actual values.
* $p < 0.05$.

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and PDE5-Is are clinically meaningful is debatable. Improvement in symptoms is poorly correlated to Q_{max} changes [11].

To further elucidate the statistically insignificant Q_{max} changes, the urodynamic effect of PDE5 inhibition on the bladder was assessed in a study of 200 men with moderate to severe BPH-LUTS with or without bladder outlet obstruction who were enrolled in an invasive and noninvasive urodynamic study. Tadalafil once daily showed no negative impact on bladder function as measured by detrusor pressure at Q_{max} or on any other urodynamic parameter assessed. The study did not proactively enroll patients with detrusor overactivity and therefore assessed only the incidence of involuntary detrusor contractions and volume at first contraction [24]. The impact of tadalafil on detrusor overactivity remains to be further investigated.

Because PDE5-Is significantly improve both BPH-LUTS and ED, it has been hypothesized that the improvement in BPH-LUTS is due to ED improvement and changes in the patient's quality of life. Several analyses have addressed whether BPH symptom improvement in clinical studies is correlated with ED symptom improvement. In a post hoc analysis from a dose-ranging tadalafil study in 716 men with ED and 340 men without, changes in BPH-LUTS after 12 wk of treatment with placebo or various doses of once-daily tadalafil were similar in men with or without comorbid ED, suggesting the improvement in BPH-LUTS was also independent of ED changes [25]. These finding were confirmed in another tadalafil study [8]. Therefore, although the mechanism by which PDE5-Is improve BPH-LUTS may share similar pathways in which PDE5-Is improve ED, these are independent of each other.

The consistent improvement in total IPSS and IPSS subscores across all PDE5 clinical studies confirm the validity of PDE5-Is as an important new class for treating BPH-LUTS. The following sections highlight the multiple mechanisms by which PDE5-Is may have an impact on the pathophysiology of BPH-LUTS.

3.2. Phosphodiesterase type 5 localization in the human outflow region (bladder, prostate, urethra)

3.2.1. Urinary bladder

Based on the hypothesis that the NO/cGMP signal pathway may play a role in the mechanism of micturition, the modulation of intracellular pathways mediated by the production of cGMP has been suggested to offer a promising possibility to achieve selective modulation of smooth musculature of the human urinary bladder. Using chromatographic methods, Truss et al. in 1996 were the first to report the presence of PDE5 in the human detrusor [26]. Immunolabeling for PDE5 was seen in smooth muscle fibers and also localized in the endothelium and the smooth muscle layer of the vesicular-deferential arteries (originating from the inferior vesical artery, the main source of blood supply to the bladder), maintaining continuous blood perfusion of the detrusor wall (Fig. 2). Given the ubiquitous expression of PDE5 in the vascular system, its presence in the bladder vascular system was expected. In contrast, the expression of PDE5 in the urothelium was only sparse [27]. At the messenger RNA level, the expression of PDE5 was also verified by reverse transcriptase polymerase chain reaction (RT-PCR) analysis [27–29]. The expression of mRNA encoding for PDE5 was higher in the detrusor and penile erectile tissue (corpus cavernosum) than in the prostate [27,29]. However, despite these findings, a pivotal role for NO and cGMP-mediated signals in the control of detrusor smooth muscle has yet to be established. Cyclic adenosine monophosphate (cAMP) regulated by PDE type 4 isoenzymes may play an even larger role in bladder smooth muscle cell relaxation.

3.2.2. Prostate

There is evidence from numerous experimental studies that the NO/cGMP system and related key proteins, including the cGMP-degrading PDE5, are pivotal players in the control of the normal function of the prostate. This may include the contractile activity of the smooth musculature, secretory glandular function, as well as the regulation of proliferation of smooth muscle, glandular epithelial cells, and stromal connective tissue [30,31]. Kuciel and Ostrowski in 1970 were the first to isolate the activity of phosphodiesterase enzymes from human prostate tissue [21]. Using ion exchange chromatography to separate proteins and an assay based on tritium-labeled cGMP, PDE5 was detected in cytosolic supernatants from minced human prostate tissue excised from the transition zone [22]. The expression of mRNA encoding for PDE5 in the prostate was later confirmed by quantitative RT-PCR [29]. However, these research approaches did not provide sufficient information on the localization of PDE5 in the prostate.

The distribution of PDE5 in different histologic portions of the prostate was revealed by immunohistochemistry: Utilization of specific antibodies demonstrated the localization of this cGMP PDE isoenzyme in glandular areas [25], the smooth musculature of the prostatic stroma, and also in blood vessels transversing the tissue sections [20,25]. As shown in Figure 2, prominent localization of PDE5 was detected in vascular (endothelial and smooth muscle) cells of human prostate. It was also shown that, in the transition zone of the prostate, PDE5 is localized in close conjunction to other key mediators of the NO/cGMP pathway. For example, in the smooth musculature, the PDE isoenzyme was found colocalized with its main substrate cGMP. The PDE5/cGMP-positive smooth muscle bundles were seen transversed by slender varicose nerve fibers immunoreactive for the neuronal isoform of NOS (nNOS). The smooth muscle fibers also presented abundant staining for the cGMP-binding, cGMP-dependent protein kinase cGKI (here cGKIß). Interestingly, abundant labeling specific for the cyclic AMP-binding protein kinase A was also registered in bundles of PDE5-immunoreactive smooth musculature. These bundles were innervated by nerve fibers containing significant amounts of vasoactive intestinal polypeptide (VIP), a neuropeptide known to promote the local production of the second messenger molecule, cAMP [32].

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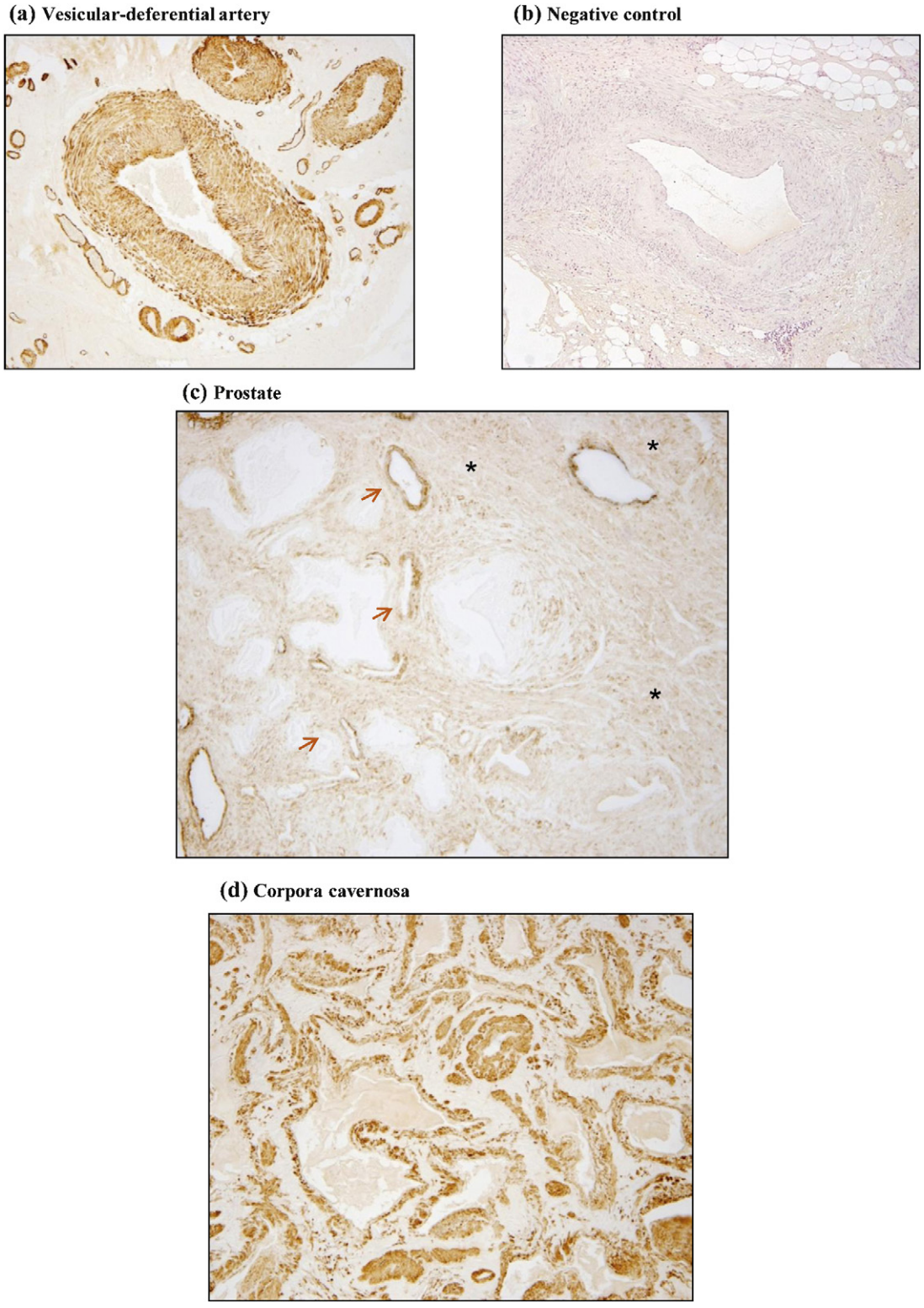

Fig. 2 – Phosphodiesterase type 5 (PDE5) expression and immunolocalization in human tissues. (a) An intense PDE5 immunopositivity was detected in the smooth muscle bundles and endothelial layer surrounding the vascular bed of transverse sections of human deferential artery. (b) Representative negative control image, hematoxylin counterstained, obtained by omitting the primary anti-PDE5 antibody in a transverse section of human deferential artery. (c) The prostatic gland section shows a scanty PDE5 immunostaining in fibromuscular stroma (asterisks), whereas it is mainly distributed in the endothelial and smooth muscle cells of blood vessels (arrows). (d) An intense PDE5 immunostaining was detected in both smooth muscle and endothelial component of corpora cavernosa section. Magnification ×4. Reproduced with permission from the International Society for Sexual Medicine [37].

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3.2.3. Urethra

It is well established that the urethra is pivotal in maintaining urinary continence and enabling coordinated micturition in both genders. Up to the present, only a very few studies have addressed the mechanisms controlling the function of human urethral smooth musculature. While the contraction of urethral smooth muscle mediated by the activation of α-adrenoreceptors has been attributed to the continence mechanism, the relaxation of the longitudinal and/or circular smooth muscle layer during micturition has been assumed to be mediated by the NO/cGMP pathway.

The functional significance of the PDE5 and other key proteins of the cGMP signaling in this process has not been clarified comprehensively. Immunohistochemistry performed on sections of human female urethra demonstrated the expression of the cGMP-specific PDE5 in vascular and nonvascular smooth muscle cells and in the vascular endothelium. In the nonvascular smooth muscle, PDE5 was found colocalized with the cGMP-binding protein kinase cGKI, whereas in vascular endothelial cells, co-staining for PDE5 and cGMP was seen [33]. More recently, the predominant expression of mRNA transcripts specifically encoding for PDE5A (cGMP-PDE) was shown by means of RT-PCR analysis [34]. Another molecular biology approach that specifically investigated the expression of the cGMP PDE5 in human LUT tissue found a consistent expression of the enzyme in the prostatic urethra. Here, the abundance of expression was higher than in the prostate gland [29]. These findings were paralleled by immunohistochemical investigations to describe the localization of PDE isoenzymes in the human male distal (penile) urethra.

In the tissue sections, PDE5-immunoreactive smooth muscle bundles were seen innervated by varicose nerve fibers characterized by the expression of nNOS; some of these nerves also presented staining specific for the VIP and calcitonin gene-related peptide [34]. Although the striated musculature seems to play a role in urethral function, no study has yet addressed the expression of PDE5 in human tissue. Using rat tissue, the expression and distribution of PDE5 was shown in striated muscle of the urethra, where it was predominantly seen colocalized to the Z-band striations. The amount of PDE5 in the striated component was six times that observed in the smooth musculature, suggesting that PDE5 is possibly significant in the regulation of striated muscles [27]. However, it remains to be clarified whether these findings can be replicated in humans and what the functional implications are.

3.3. Smooth muscle relaxation

The issue of how an enhancement of the cGMP pathway—for example, by inhibiting the activity of PDE5—can affect the relaxation of LUT smooth musculature has been investigated in various in vitro tissue bath studies typically using nonmalignant isolated surgical specimens of the urinary bladder (including both the detrusor and bladder neck), prostate, or urethra. The experimental models have applied agents such as muscarinic (carbachol) or adrenergic receptor agonists (norepinephrine, phenylephrine) known to contract effectively the respective tissues, as well as various PDE5-Is and the NO donor drug sodium nitroprusside (SNP), characterized as a reference drug to stimulate the production of cGMP via activation of the enzyme guanylyl cyclase.

3.3.1. Urinary bladder

When considering the effect of PDE5-Is on the human urinary bladder, it is important to differentiate between bladder dome and bladder neck smooth musculature. Regulation of human bladder smooth muscle by the NO/cGMP pathway differs markedly according to the region of the bladder studied. Early studies using PDE5-Is and square-shaped strips of human detrusor smooth muscle (full wall specimens devoid of the urothelium) challenged by the muscarinic agonist carbachol did not present striking evidence supporting a role of PDE5 in the control of bladder function: The relaxant responses of the tissue to the cumulative addition (0.01–200 μM) of the PDE5-Is zaprinast and dipyridamole were determined as <20% [26]. Oger et al. investigated the effect of sildenafil (10 nM–30 μM) on the tonic contraction of human bladder dome smooth muscle in response to carbachol [28]. Sildenafil exerted a direct relaxant effect; however, high concentrations of the PDE5-I were needed. The relaxant effect involves the cGMP pathway as well as the activity of K^+ channels. Because the relaxation remained unaltered in the presence of SNP, it was assumed that NO only makes a minor contribution to the relaxation induced by sildenafil [35]. Thus, in the human bladder dome, the effects of PDE5-Is are moderate and may not completely explain the improvement of urinary storage symptoms observed in patients treated with PDE5-Is.

In the human bladder neck, the nitrergic innervation is more prominent, and thus the effects of PDE5-Is are different. In isolated human (male) bladder neck strips, SNP induced a mediocre relaxation (maximum: 37% ± 4%) of preparations precontracted with carbachol. This relaxation increased significantly (to 62% ± 3%) following preexposure of the tissue to a threshold concentration of the PDE5-I vardenafil (100 nM) [27]. Similar findings were reported with sildenafil where a relaxing effect was shown on isolated human bladder neck precontracted with phenylephrine [36]. It was also shown in experiments using human vesicular-deferential arteries that tadalafil increased the relaxant response to SNP [37].

3.3.2. Prostate

Uckert et al. conducted tissue bath studies using smooth muscle isolated from the periurethral and transition zones of nondiseased human prostates [22]. A dose-dependent reversal was seen of the tension induced by norepinephrine in response to sildenafil and zaprinast (1 nM–10 μM) [23]. However, the efficacy of these compounds did not exceed 30% reversal of the initial contractile force generated by the tissue preparations in response to norepinephrine. In another experimental sequence using the same in vitro setup, the contraction of the tissue was also antagonized by tadalafil (mean: −52% reversal of tension) and vardenafil

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(mean: −35%) [23]. PDE5-Is might also interfere with the contraction of prostatic smooth muscle mediated by the release of endogenous peptides because tadalafil could also reduce prostate contractions induced by endothelin-1 [38].

It was also shown that PDE5-Is tend to be more effective in vitro in the presence of a threshold concentration of sodium nitroprusside known to stimulate the activity of the soluble guanylyl cyclase (sGC) [39]. This is due to a synergistic effect that is likely to result from combining both an enhanced local tissue production of cGMP by NO via the sGC and blockade of the breakdown of the second messenger by PDE5-Is.

3.3.3. Urethra

In the human urethra, large amounts of NOS-containing nerves have been demonstrated in the muscular wall, around blood vessels close to the urothelium, as well as in the sarcolemma of intramural striated muscle fibers of the membranous urethra [40]. PDE5-immunoreactive smooth muscle bundles innervated by varicose nerve fibers characterized by the expression of nNOS, the calcitonin gene-related peptide, and VIP were also seen [34]. In preliminary organ bath experiments, the contraction induced by noradrenaline of isolated human female urethra was almost completely reversed in response to 10 μM sildenafil; the reversal of the tension brought about by tadalafil (30 μM) was 47% [33]. In a similar experimental setup using specimens of male proximal penile urethra, the adrenergic tension of the tissue was antagonized by 35%, 26%, and 20% following the application of 10 μM sildenafil, vardenafil, or tadalafil, respectively. The relaxation effects were paralleled by a several-fold elevation in cGMP [41]. An inhibition of the contraction induced by phenylephrine of muscle strips of the prostatic urethra obtained from male New Zealand rabbits in response to the PDE5-I udenafil was also shown. Udenafil relaxed the urethral strip preparations in a dose-dependent manner, with a maximum relaxation at the final drug concentration (1 mM) of 44% [42]. In contrast, in another study, the maximum contraction response obtained through electrical field stimulation of circular segments of guinea pig urethra was not altered by pretreatment with a high concentration of SNP (100 μM). In tissue specimens challenged by noradrenaline, the relaxation observed at 10 Hz was not attenuated in the presence of the NO synthase inhibitor N^G-nitro-L-arginine. Thus it remains unclear whether the relaxation of urethral smooth muscle depends on the activity of NO and cGMP [43].

3.4. Increased blood perfusion

A growing body of epidemiological studies documented a strong and independent relationship between BPH-LUTS and metabolic syndrome, which is essentially characterized by a syndromic clustering of cardiovascular and metabolic alterations [44]. Research in animal models of LUTS indicated that either systemic hypertension [37,45] or metabolic derangement [46–48] can lead to morphologic/structural alterations of both prostate and bladder including fibrosis, increased contractile activity, and urethral resistance, having chronic ischemia as a common feature. Hence the view that pelvic ischemia/hypoxia might underlie LUTS is emerging. Blood supply to the LUT (lower part of the bladder, the prostate, and the seminal vesicles) is provided by small branches of the inferior vesical artery, which frequently arises in common with the middle rectal artery from an anterior division of the internal iliac artery [49]. Interestingly, the human vesical deferential artery is enriched in PDE5, showing mRNA levels and cGMP hydrolyzing activity comparable with corpora cavernosa [37]. In addition, blood vessels within the prostate and bladder are widely positive for PDE5 expression, localized in the endothelial and smooth muscle cells [29]. Pelvic vasculature might therefore represent a new target for PDE5-Is. Accordingly, in a rat experimental model, it was recently demonstrated that PDE5 actively regulates blood supply to the LUT [37,45]. Spontaneous hypertensive rat (SHR) is a rat strain characterized by a reduced pelvic blood flow to the genitourinary tract when compared with its normotensive counterpart, Wistar-Kyoto rats (WKY) [50]. By injecting these rats intraperitoneally with the bioreductive drug pimonidazole hydrochloride (Hypoxyprobe), it is possible to visualize hypoxic cells within LUT by immunohistochemistry. Hypoxyprobe is a water-soluble substituted 2-nitrominidazole that forms adducts with proteins in cells that are at an oxygen pressure $\leq$10 mm Hg. Prostate and bladder of SHR rats are definitively more hypoxic than in WKY rats, with a predominant distribution of hypoxic cells in the epithelial layers. Both acute (vardenafil 10 mg/kg, 90 min before death; see Fig. 3: rat bladder from Morelli et al. [45]) and chronic (tadalafil 2 mg/kg per day for 1, 7, and 28 d; see Fig. 4: rat prostate from Morelli et al. [37]) PDE5 blockade restored LUT oxygenation in SHR rats up to the level observed in WKY rats. A 2009 study, performed using contrast-enhanced ultrasound in 12 BPH patients awaiting surgery, demonstrated increased prostatic blood perfusion following administration of 20 mg tadalafil [51]. These preliminary findings indicate that PDE5-Is might relax LUT vasculature and consequently increase blood supply and tissue oxygenation, therefore possibly ameliorating urinary function and reduce BPH-related LUTS. The fact that LUTS are significantly reduced within 1–2 wk in clinical trials suggests that other factors than increased blood perfusion play a role.

3.5. Afferent nerve activity

The NO/cGMP pathways are believed to play important roles in the function of the nervous system in the LUT. nNOS is expressed in locations including the uroepithelium and nerves innervating the bladder neck and urethra; endothelial NOS is present in vascular endothelium [52,53]. Enzymatic activity (NOS) has been measured from various regions including the bladder neck and prostatic urethra [54]. Guanylate cyclase has been detected in afferent nerves and interstitial cells with the highest levels found in the urethra. Increases in interstitial cell number and connectivity have been demonstrated in animals following experimental spinal cord injury (SCI) [55,56], which results in augmented pacemaker activity in these cells. This type of activity, in turn, may drive intrinsic smooth muscle

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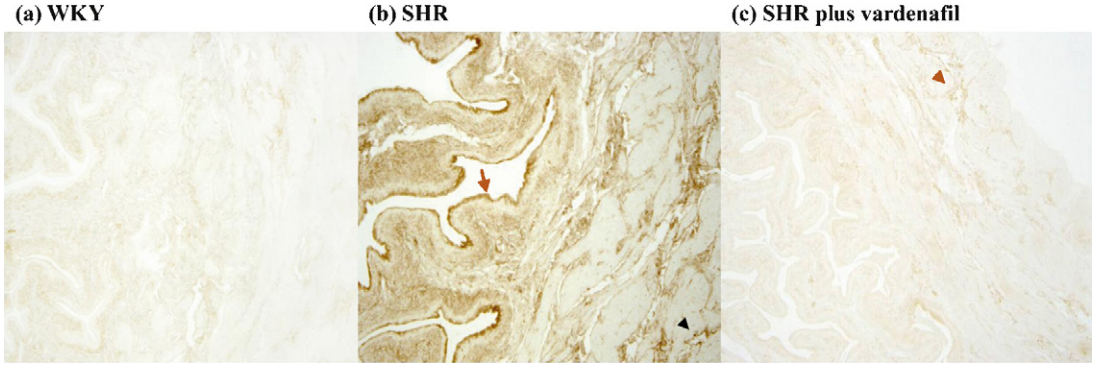

Fig. 3 – Immunohistochemical staining of Hypoxyprobe in rat bladder. Hypoxyprobe-positive protein adducts were revealed in hypoxic cells (PO_2 <10 mm Hg) of bladder transverse sections by a monoclonal antibody (magnification ×10). (a) Wistar-Kyoto rats (WKY): Only scanty positive labeling is present. (b) Untreated spontaneously hypertensive rats (SHR): Massive hypoxia is present in both the urothelium/suburothelium (arrows) and vascular endothelium (arrowheads). (c) Vardenafil-treated spontaneously hypertensive rats (SHR). Hypoxyprobe labeling is dramatically decreased; only a few endothelial (arrows) cells are positive. Reproduced with permission from the International Society for Sexual Medicine [45]. WKY = Wistar-Kyoto rats.

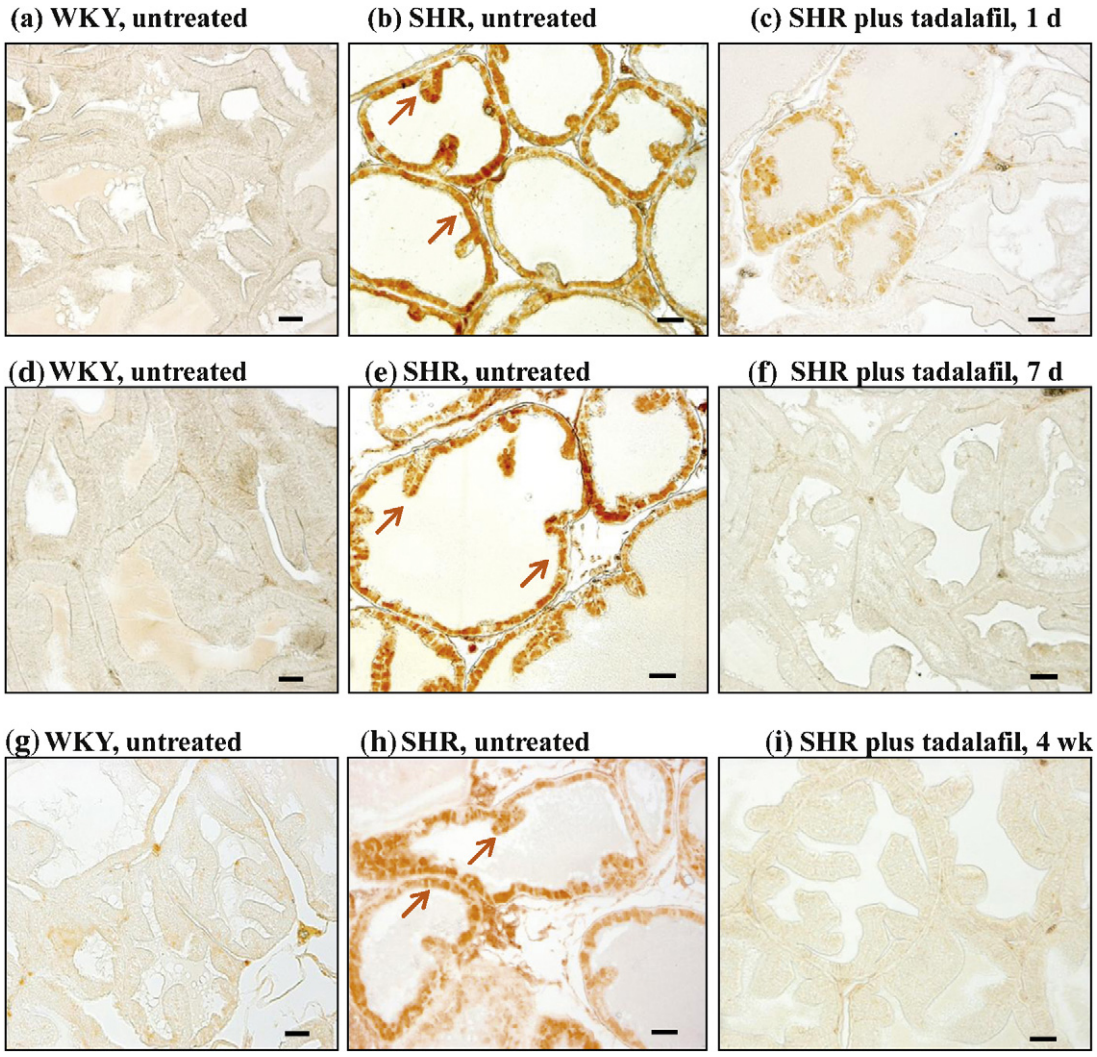

Fig. 4 – Immunohistochemical staining of Hypoxyprobe in rat prostate. Hypoxyprobe-positive protein adducts were revealed in hypoxic cells (PO_2 <10 mm Hg) of prostate transverse sections by a monoclonal antibody (magnification ×10). Images (magnification ×10; bars = 50 mm) are representative of each experimental group at each time point (a–c: 1 d; d–f: 7 d; g–i: 28 d). The staining was almost undetectable in WKY prostate sections (a, d, g). The prostate sections from untreated SHR were strongly immunopositive for Hypoxyprobe in the epithelial layers (arrows) surrounding prostate ducts, which were also characterized by alveolus dilation and reduction of interstitial and stromal spaces (b, e, h). Prostate sections from tadalafil-treated SHR show a net reduction of Hypoxyprobe immunopositivity after (c) 1 d, becoming absent after both (f) 7 d and (i) 28 d. Reproduced with permission from the International Society for Sexual Medicine [37].

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contractions, thereby stimulating afferent firing [57]. Beside afferent nerves, the urothelium (and also urethral epithelium) are able to synthesize and release NO in response to a number of stimuli [58,59]. Release of NO may in part act to uncouple cells (urothelial, interstitial) preventing this type of pacemaker activity.

PDE5-Is are thought to decrease the perception of bladder filling, thereby reducing the sensation of urgency. The mechanism may involve a reduction in the release of neuropeptides and activity of afferent nerves via activation of the NO/cGMP pathway [60,61]. In the urinary bladder, the NO/cGMP pathway can have a number of functional roles. Both the production of NO as well as the expression levels of enzymes that synthesize NO can be altered by a variety of manipulations and/or pathophysiologic mechanisms. For example, inhibition of NO can result in bladder hyperactivity and decrease in bladder capacity [62]. Chronic NO deficiency [63] or scavenging of NO via use of intravesical oxyhemoglobin [64] has been shown to result in bladder overactivity or hyperactivity to various stimuli. This finding may be due to changes in levels of NO acting at a number of sites including bladder afferents, interstitial cells, as well as smooth muscle, resulting in alterations in bladder activity.

Only a limited number of studies address the impact of PDE5 inhibition in sensory function, and differences between species and the influence of pathology can make interpretation difficult. The impact on bladder hyperactivity was assessed by Caremel and colleagues in anesthetized female Sprague-Dawley rats that were continuously perfused with capsaicin and then challenged with an NO donor (SNP), a cGMP analog (8Br-cGMP), or a PDE5-I (either sildenafil or vardenafil) [65]. The addition of NO or PDE5-Is increased the intercontraction interval and micturition pressure threshold, suggesting that the NO/cGMP signaling pathway exerted an inhibitory effect on bladder afferent activity [65]. Minagawa and colleagues recently examined the impact of increasing doses of intravenous tadalafil on bladder afferent nerve activity caused by bladder distension and acrolein-induced hyperactivity in female Sprague-Dawley rats [66]. Bladder nerve afferents were identified by electrical stimulation of the pelvic nerve and by bladder distension, and divided by conduction velocity into myelinated Aδ- or unmyelinated C-fibers. Tadalafil significantly decreased the single afferent activity of both the Aδ- and C-fibers in response to bladder filling and urothelial bladder-installation irritation without affecting the bladder tone [66]. SCI rats mimic the voiding pattern of patients with neurogenic detrusor hyperactivity due to SCI by displaying nonvoiding contractions (NVCs) during bladder filling. NVCs in SCI rats are associated with bladder afferent fiber hyperexcitability [67,68].

When Behr-Roussell and colleagues examined the impact of PDE5 inhibition with vardenafil in SCI rats, they found a significant reduction in bladder afferent nerve firing during bladder filling and a significant decrease in amplitude of NVCs [69]. In a similar animal model, Sasatomi and colleagues found that increased NO level caused by the inhibition of arginase, which degrades L-arginine into L-ornithine, decreased NVC through reduced afferent nerve activity [70]. Taken together, these studies show support for NO/cGMP pathway and a MOA for PDE5 inhibition in the reduction of afferent nerve activity in both bladder physiology and pathophysiology.

4. Conclusions

PDE5 isoenzymes are highly expressed in human LUT tissues. PDE5 inhibition results in smooth muscle relaxation and increased pelvic blood perfusion in these tissues and likely modulates afferent nerve activity. This activity may affect nonvascular or vascular smooth muscle tone. We propose that the improvement in both storage and voiding urinary symptoms observed after 1–2 wk of tadalafil could be caused by the smooth muscle cell relaxation in bladder neck, prostate, and urethra, with the maintenance of effect possibly supported by the smooth muscle cell relaxation of these organs' vascular supply and increased blood perfusion and oxygenation. Modulation of the sensory output from the LUT is likely to play a role in both the short and long term.

Author contributions: François Giuliano had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Viktrup, Kissel.
Acquisition of data: Giuliano, Ückert, Maggi, Birder, Kissel, Viktrup.
Analysis and interpretation of data: Giuliano, Ückert, Maggi, Birder, Kissel, Viktrup.
Drafting of the manuscript: Giuliano, Ückert, Maggi, Birder, Kissel, Viktrup.
Critical revision of the manuscript for important intellectual content: Giuliano, Ückert, Maggi, Birder, Kissel, Viktrup.
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