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REVIEW ARTICLE

PDE5 inhibitors – pharmacology and clinical applications 20 years after sildenafil discovery

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The discovery of the nitric oxide/cGMP pathway was the basis for our understanding of many normal physiological functions and the pathophysiology of several diseases. Since the discovery and introduction of sildenafil, inhibitors of PDE5 have been the first-line therapy for erectile dysfunction (ED). The success of sildenafil in the treatment of ED stimulated research in the field of PDE5 inhibition and led to many new applications, such as treatment of lower urinary symptoms, and pulmonary arterial hypertension, which are now approved indications. However, PDE5 inhibitors have also been used in several other disorders not discussed in this review, and the fields of clinical use are increasing. In the present review, the pharmacological basis of the NO/cGMP pathway and the rationale and clinical use of PDE5 inhibitors in different diseases are discussed.

Abbreviations

ED, erectile dysfunction; LUTS, lower urinary tract symptoms; NANC, non-adrenergic non-cholinergic; PAH, pulmonary arterial hypertension; PKG, cGMP-dependent protein kinase

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Introduction

The discovery in the late 1980s of **nitric oxide** as the endothelium-derived relaxing factor was the key to understanding the **NO-soluble GC (sGC)-cGMP** pathway. This discovery led to intensive research on the physiology, pharmacology and pathophysiology of NO (Moncada *et al.*, 1991; Denninger and Marletta, 1999; Murad, 2006), which resulted in Furchgott, Ignarro and Murad receiving the Nobel Prize for Physiology or Medicine in 1998.

NO mediates its biological effects by activating sGC and increasing cGMP synthesis which, in turn, activates certain proteins resulting in different actions. cGMP actions are terminated by **PDE5** and PDE5 inhibitors, such as **zaprinast** and **sildenafil**, proved vital in the elucidation of the widespread role of cGMP in nitrergic transmission. The synthesis of sildenafil and the (serendipity) discovery of its effects on penile erection provided a major breakthrough in the treatment of erectile dysfunction (ED) and opened new fields of clinical application for this class of drug. Although many PDE5 inhibitors have since been synthesized and developed, most of the information available is for sildenafil, **tadalafil** and **vardenafil**.

Literature search

A literature search of the electronic sources of different databases such as PubMed Central, Google Scholar and Scopus was used. Keywords used for search included, but were not limited to, PDE5, PDE5 inhibitors, NO, cGMP, ED, male lower urinary tract symptoms (LUTS) and pulmonary arterial hypertension (PAH). In addition, articles from previous reviews on NO, ED and male LUTS by the author were restudied. Sixty articles, considered to cover the field, were selected and referred to in this review.

The NO/soluble GC/cGMP pathway

The NO/sGC/cGMP pathway has been described in detail elsewhere (Moncada *et al.*, 1991; Denninger and Marletta, 1999; Murad, 2006) (Figure 1). NO is synthesized by the oxidation of L-arginine catalysed by **NOS** that utilizes NADPH and O_2 as substrates. Three isoforms of NOS have been identified: **neuronal NOS** (nNOS or NOS1), **inducible NOS** (iNOS or NOS2) and **endothelial NOS** (eNOS or NOS3). NO mediates its biological effects by activating sGC and increasing cGMP synthesis from GTP. The cGMP formed activates **cGMP-dependent protein kinase (PKG, cGK)**, which, in turn, activates certain proteins resulting in different effects on, for example, growth, viability, smooth muscle relaxation, secretion, ion transport, endothelial permeability and gene transcription. cGMP's effects are terminated by PDE5, which breaks down its phosphodiester bond, an effect prevented by PDE5 inhibitors. It should be noted that the effects of PDE5

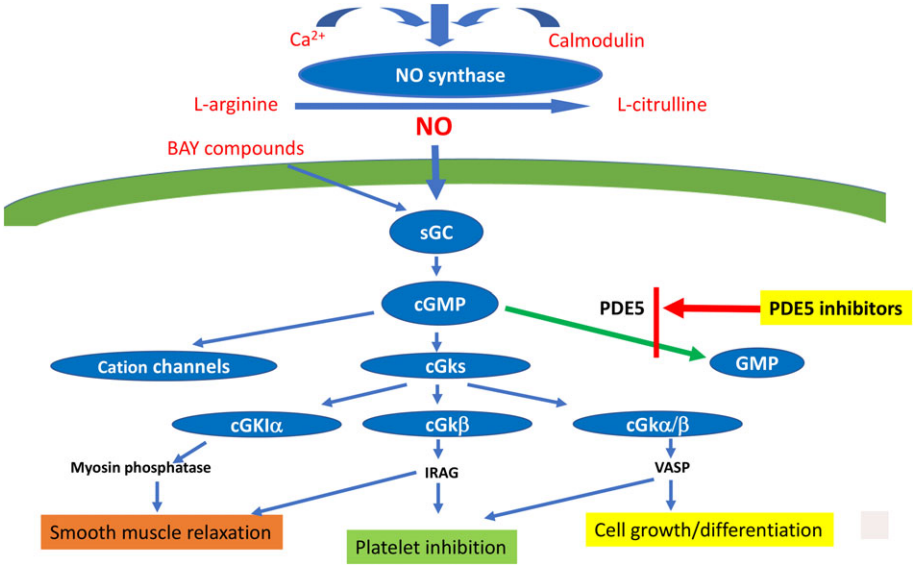

Figure 1

Cellular signalling with NO and cGMP (modified from Murad, 2006). NO is synthesized by the oxidation of L-arginine catalysed by NOS. NO mediates its biological effects by activating sGC and increasing cGMP synthesis from GTP. cGC can also be activated by non-NO-dependent activators and stimulators (BAY compounds): NO-independent, haem-dependent stimulators of sGC (BAY 41–2272, BAY 41–8523, BAY 63–2521 and BAY 60–4552) and NO-independent, haem-independent sGC activators (HMR 1766, BAY 58–2667 and BAY 60–2770). The cGMP formed activates cation channels but also cGMP-dependent protein kinases (cGKs), which, in turn, activate certain proteins (myosin phosphatase, inositol 1,4,5-trisphosphate receptor I (IP(3)RI)-associated protein = IRAG, and vasodilator-stimulated phosphoprotein = VASP), resulting in, for example, smooth muscle relaxation, platelet inhibition and cell growth/differentiation. THe effects of cGMP are terminated by PDE5, which breaks down its phosphodiester bond, an effect prevented by PDE5 inhibitors. It should be emphasized that the effects of PDE5 inhibitors are crucially dependent on the activity of the NO/GC/cGMP pathway.

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As indicated in Figure 1, sCG can also be influenced by drugs ('BAY compounds') that are able to act directly on the enzyme in an NO-independent manner (Mónica and Antunes, 2018). Two classes of drugs have been developed to treat cardiovascular diseases: NO-independent, haem-dependent *stimulators* of sGC (BAY 41–2272, BAY 41–8523, BAY 63–2521 and BAY 60–4552) and NO-independent, haem-independent sGC *activators* (HMR 1766, BAY 58–2667 and BAY 60–2770). Some of these drugs, which are in clinical use [e.g. **riociguat** (BAY 63–2521)], may also be used for urogenital disorders (Mónica and Antunes, 2018).

Historical background of PDE5 inhibitors – discovery of sildenafil

Zaprinast was synthesized in 1974 and later characterized as the first selective PDE5 inhibitor (Gibson, 2001). Even though later studies revealed that zaprinast was not selective for PDE5, it became an important tool for inhibiting PDE5. Thus, zaprinast was shown to enhance NO-induced relaxation of isolated corpus cavernosum from animals (Ignarro *et al.,* 1990) and humans (Rajfer *et al.,* 1992).

When exploring PDE5 as a target for a range of cardiovascular disorders, particularly hypertension and angina pectoris, Terrett *et al*. (1996) found that PDE5 was the predominant hydrolysing enzyme in the cytosolic fraction from human corpus cavernosum and suggested that one of their inhibitors synthesized, sildenafil, which was a potent and highly selective PDE5 inhibitor, could be useful as an orally active treatment for male ED. That this was the case was confirmed in a double-blind, randomized, placebo-controlled, crossover study on 12 patients (aged 36–63 years) with ED (Boolell *et al.,* 1996), and this initiated rapid progress in the development of selective PDE5 inhibitors for this indication (Andersson, 2001; 2011). Since the introduction of sildenafil, the approved indications for PDE5 inhibitors include not only ED but also male LUTS and PAH. However, PDE5 inhibitors have also been used to treat an increasing number of conditions, but with varying degrees of effectiveness (Gur *et al.,* 2012; Ribaudo *et al.,* 2016).

Distribution of PDE5 enzymes

PDE5 isoenzymes have now been identified in a wide variety of tissues, both in animals and man. They have been demonstrated in, for example, the smooth muscle cells of the corpus cavernosum, vascular and visceral smooth muscle, skeletal muscle, platelets, kidney, lung, spinal cord, cerebellum, pancreas, prostate, urethra and bladder (Lin *et al.,* 2006; Francis *et al.,* 2011; Uckert and Kuczyk, 2011).

Eleven different **PDE families** (PDE 1–11) have been described (Francis *et al.,* 2011; Keravis and Lugnier, 2012). More than 50 isoforms have been characterized, all differing in their primary structures, specificity for cAMP and cGMP, cofactor requirements, kinetic properties, mechanisms of regulation and tissue distributions (Francis *et al.,* 2011). Some PDEs are selective for the hydrolysis of cAMP (PDE 4, 7 and 8) or cGMP (PDE 5, 6 and 9), while others can hydrolyse both cAMP and cGMP (PDE1, 2, 3, 10 and 11). Two PDE5 isoforms, PDE5A1 and PDE5A2, have been demonstrated in humans, but a third isoform, PDE5A3, has also been identified. The variants may allow for differential control of PDE5A gene expression in various cells. The three human PDE5 isoforms differ only in the 5′ end of the mRNA and the corresponding N-terminal of the protein. PDE5A1, PDE5A2 and PDE5A3 share similar cGMP-catalytic activities that are differently inhibited by sildenafil or zaprinast, with PDE5A1 being more resistant to such inhibition than PDE5A2 or PDE5A3. PDE5A1 and PDE5A2 are found in nearly all tissues, but PDE5A2 is more widespread, and the distribution of PDE5A3 is limited to smooth muscle (Lin *et al.,* 2006).

General pharmacology of PDE5 inhibitors

As mentioned, inhibition of the PDE5 isoenzymes results in intracellular accumulation of cGMP, which activates cGMP-dependent protein kinase with subsequent phosphorylation of specific substrate proteins. As a second messenger, cGMP plays a central role in signal transduction and regulates a number of physiological responses, such as smooth muscle relaxation, platelet and cardiac functions through a number of downstream mechanisms (Murad, 2006; Stasch *et al.,* 2011).

Relaxation of smooth muscle

It is well established that NO, **atrial natriuretic peptide** and several other endogenous vasodilators regulate smooth muscle tone through activation of sGC, elevation of cGMP and activation of PKG (cGK), which in turn phosphorylates several proteins. Three different PKGs (PKGIa, PKGIb and PKGII) have been identified in mammals (Figure 1). The NO/cGMP effects on contraction in smooth muscle appear to be mediated specifically by PKG, but not **PKA**, because inactivation of PKGI in mice abolished both NO/cGMP-dependent relaxation of vascular and intestinal smooth muscle and inhibition of platelet aggregation, causing hypertension, intestinal dysmotility and abnormal haemostasis (Pfeifer *et al.,* 1998). There are several specific physiological substrates for PKG in smooth muscle including the regulatory myosin-binding subunit of myosin phosphatase, calcium-activated maxi K^+ (BK_{Ca}; **K_{Ca}5.1**) channels and IRAG (IP3 receptor-associated cGMP kinase substrate). Phosphorylation of all of these targets contributes to a reduction in intracellular Ca^{2+} concentration or reduction in sensitivity to Ca^{2+} and thereby decreased smooth muscle tone.

Platelet aggregation

The role of the NO/cGMP pathway for platelet function is well established (Mellion *et al.,* 1981; Radomski *et al.,* 1990; Smolenski, 2012). **Prostacyclin (PGI_2)** and NO, released from the endothelium, are potent inhibitors of platelet function. Their actions are mediated by both PKA and PKG synthesizing cAMP and cGMP respectively. Platelets have been shown to express several PDEs: PDE2A, PDE3A and PDE5A. These three isozymes account for the majority (more than 90%) of platelet PDE activity. PDE2 and PDE3 are able to

degrade both cAMP and cGMP; however, they mainly regulate cAMP degradation, whereas PDE5 specifically degrades cGMP. PDE5 is an important regulator of platelet function (Rondina and Weyrich, 2012); knockout of the PKGI gene in mice led to a prothrombotic phenotype. The main isoform expressed in human platelets is PKGIb. In platelets of PKGI knockout mice, the effects of a cGMP analogue on platelet shape change, granule release and aggregation were abolished, whereas the effects of a cAMP analogue were maintained (Pfeifer *et al.,* 1998).

PDE inhibitors in clinical use for stroke prevention (dipyramidole, **cilostazol**) mainly act *via* cAMP, even if they also increase cGMP levels (Rondina and Weyrich, 2012; Yeung and Holinstat, 2012). However, PDE5 inhibitors used to treat ED, including sildenafil, vardenafil and tadalafil, potentiate the effect of nitrovasodilators on platelets. Sildenafil and tadalafil accumulated in platelets with an up to fourfold higher accumulation when platelets were pretreated with an NO donor (Bajraktari *et al.,* 2017). Sildenafil also prolonged bleeding time in healthy volunteers after acute administration (Berkels *et al.,* 2001).

Cardiac function

Both NOS1 and NOS3 are constitutively expressed in the cardiovascular system. NOS3 is mostly found in coronary vascular and endocardial endothelial cells and, to a lesser extent, in cardiac myocytes, whereas NOS1 is predominantly localized to the sarcoplasmic reticulum. In the myocardium, constitutive NO production affects the function and phosphorylation state of several proteins that are involved in excitation-contraction coupling, for example, the **L-type Ca^{2+} channel**, troponin I and phospholamban, and inhibits both oxygen consumption and **β-adrenoceptor** mediated inotropy; abnormal NOS signalling plays a key role in many cardiac disorders (Carnicer *et al.,* 2013; Kim and Kass, 2017). An important hallmark of cardiac failure is abnormal second messenger signalling due to impaired synthesis and catabolism of cAMP and cGMP. Experimental findings using NO donors and NOS inhibitors, or gene deletion, clearly implicate dysfunctional NOS as a critical contributor to many cardiovascular disease states, including diabetic cardiomyopathy, ischaemia/reperfusion injury and atrial fibrillation (Simon *et al.,* 2014). The regulation of cardiac functions by endogenous NO has been suggested to be dependent on the distinct subcellular locations of nNOS and eNOS, through vascular-dependent and vascular-independent effects (Massion and Balligand, 2003). The heart expresses seven of the 11 major PDE sub-types – PDE1, 2, 3, 4, 5, 8 and 9 (Kim and Kass, 2017) – and their different effects on cAMP and cGMP signalling in various cell types, including cardiomyocytes, provides intriguing therapeutic opportunities to counter heart disease. An abundant expression of PDE5 has been demonstrated in isolated canine or murine ventricular cardiomyocytes (Das *et al.,* 2005), and PDE5 is highly expressed in both experimental and human heart disease. There should thus be possibilities to influence cardiac function by PDE5 inhibition, and there is currently a tremendous interest in identifying new clinical uses of PDE5 inhibitors for treatment of a variety of cardiovascular diseases (Kim and Kass, 2017; Sevil Korkmaz-Icöz *et al.,* 2018).

Approved clinical applications of PDE5 inhibitors

There are currently three approved indications for PDE5 inhibitors: ED, LUTS and PAH.

General rationale for use of PDE5 inhibitors

In the *corpora cavernosa*, relaxation of the smooth muscle of the sinusoids is induced by NO release from endothelial cells and nitrergic neurons surrounding the arteries and sinusoids (Figure 2). By inhibiting PDE5 hydrolytic activity, PDE5 inhibitors produce a higher rate of accumulation of cGMP in response to the NO, thus enhancing the erectile response.

In the LUT, there is an abundance of PDE5 isoenzymes and their inhibition reduces smooth muscle cell proliferation, relaxes smooth muscle cells, increases tissue oxygenation and modulates afferent nerve activity (Figure 3).

In the pulmonary vasculature, NO plays an important role as a vasorelaxant, and a high level of PDE5 has been demonstrated (Figure 4). This provides a strong molecular basis for using a PDE5 inhibitor to treat PAH.

Erectile function and dysfunction

Historical background. To maintain the penis in the flaccid state, the smooth muscles of penile arteries and trabeculae are kept contracted, probably by the release of noradrenaline (NA) acting on postjunctional **α_1-adrenoceptors**. Dilatation of penile arteries and sinusoids with the resultant increase in penile blood flow is considered a primary haemodynamic event in erection (Figure 2). Stimulation of the parasympathetic sacral nerves innervating the penis produces erection, and several findings have suggested that **acetylcholine** participates in the induction of erection (Andersson and Wagner, 1995; Andersson, 2001). However, experiments on isolated erectile tissues showed that muscarinic receptor stimulation had no or only minor inhibitory effects on relaxations induced by electrical field stimulation, and the fact that **atropine** was without effect on erections provoked by visual stimulation or by local vibration (Wagner, 1981) favoured the occurrence of an non-adrenergic non-cholinergic (NANC) dilator substance. The finding of Furchgott and Zawadzki (1980) that acetylcholine-induced dilatation of rabbit aorta was mediated by the release of a relaxant agent from the endothelial cells, which they later termed the endothelium-derived relaxing factor, focused interest on NANC mechanisms in penile erectile tissue. The reaction of penile erectile tissue to different autonomic drugs and electrical field stimulation was a starting point for the development of drugs that could influence penile erection. Hedlund and Andersson (1985) found that in NA-contracted human corpus cavernosum preparations acetylcholine, but particularly the muscarinic receptor agonist, **carbachol**, had a potent relaxant action, and Saenz de Tejada *et al.* (1988) showed that the relaxing effect of exogenous acetylcholine is dependent on an intact endothelium. Subsequent studies have indicated that other agents beside acetylcholine also cause vasodilatation by an endothelium-dependent mechanism, but that the most important relaxing factor is NO. This was shown in both rabbit (Ignarro *et al.,* 1990) and human (Holmquist *et al.,*

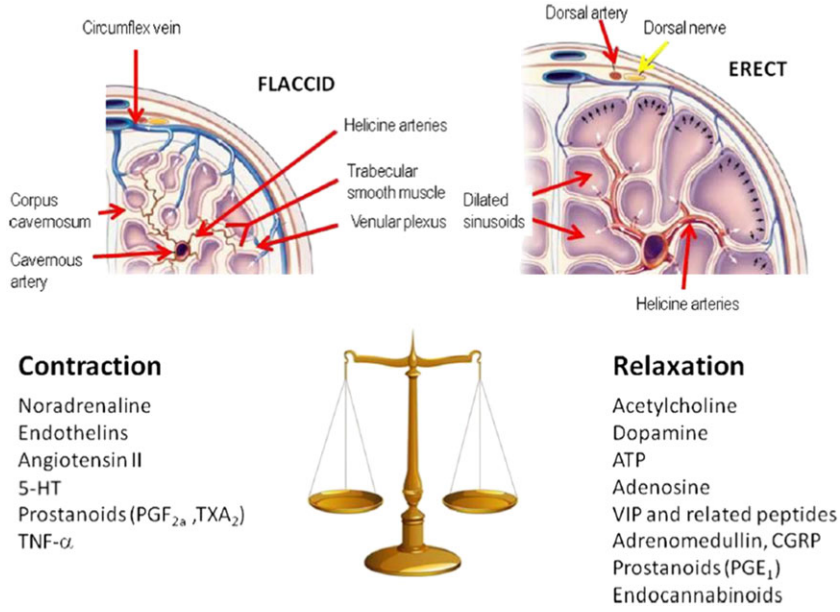

Figure 2

Mechanisms of penile erection (from Andersson, 2011). In the penile vessels and the smooth muscle of the corpora cavernosa, the balance between contractile and relaxant factors controls the degree of tone of the penile vasculature and of the smooth muscle. This in turn determines the functional state of the penis: detumescence and flaccidity, tumescence and erection. NO is released from nitrergic nerves, from the endothelium in response to the release of acetylcholine by parasympathetic endothelial nerve endings and by the shear stress produced by increased blood flow in the corporeal sinusoids. This dilates the helicinal arteries, fills the sinusoids, which eventually compresses the circumflex veins against the tunica albuginea, finally resulting in erection.

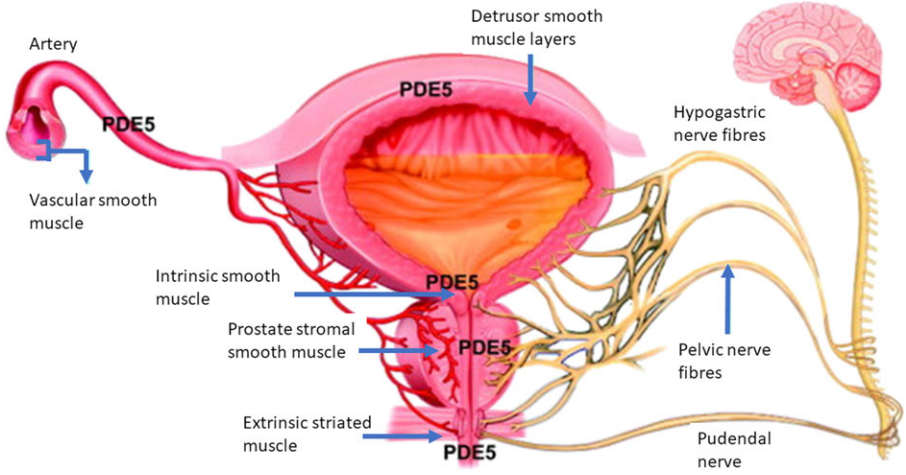

Figure 3

Distribution of PDE5 enzymes in the lower urinary tract. PDE5 isoenzymes are widely distributed in the LUT: the smooth muscle of the detrusor and the internal urethral sphincter, prostatic stroma, external urethral sphincter and the vasculature. Each of these locations can be involved in the pathophysiology of LUTS, resulting in afferent and efferent signalling in the pelvic, hypogastric and pudendal nerves. PDE5 inhibitors may improve LUTS by improving LUT oxygenation, relaxation of smooth muscle, negative regulation of proliferation and trans-differentiation of prostate stroma, down-regulation of prostate inflammation and reduction of bladder afferent nerve activity.

1991, 1992; Rajfer *et al.*, 1992) corpus cavernosum. These studies and the observation that the PDE5 inhibitor, sildenafil, caused penile erection were the basis for the development and use of number of selective PDE5 inhibitors for treatment of ED.

Physiology of penile erection. Sexual arousal through visual, tactile, olfactory or imaginative stimuli stimulates neural pathways in the brain that result in the release of NO from nerves and endothelial cells directly into the penis (Andersson and Wagner, 1995; Andersson, 2001, 2011).

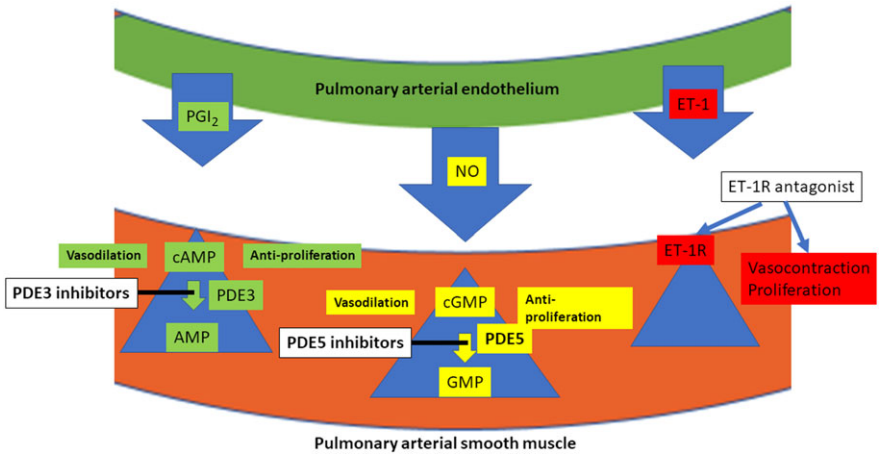

Figure 4

The principal targets for current therapies for PAH PGI_2 (prostacyclin), relaxes smooth muscle cells *via* increases in intracellular cAMP levels. Inhibitors of PDE3, such as milrinone, stabilize the cAMP concentrations. NO dilates smooth muscle cells by increasing intracellular cGMP levels, and these levels are maintained by inhibition of PDE5 by, for example, sildenafil. For PDE5 inhibitors to be effective, there must be activity in the NO/sGC/cGMP pathway. ET-1 is a vasoconstrictor, and blockers of both **ET_A** and **ET_B** receptor subtypes, such as **bosentan**, decrease smooth muscle tone.

Erectogenic stimuli may also originate in the sacral spinal erection centre in response to direct tactile stimulation of the penis, and efferent neurons from this centre reach the pelvic plexus, from which postganglionic nitrergic fibres pass in the cavernous nerves to the corpora cavernosa. NO is released from nitrergic nerve terminals in the corpus cavernosum and also from the endothelium in response to the release of acetylcholine by parasympathetic endothelial nerve endings and by the shear stress produced by increased blood flow in the corporeal sinusoids. NO penetrates into the cytoplasm of smooth muscle cells and binds to the haem moiety of sGC. The interaction of NO with sGC causes a conformational change in this enzyme, activating it, and this will catalyse the breakdown of GTP into cGMP which is the intracellular trigger for penile erection. cGMP activates PKG leading to a cascade of events. Even if other pathways with cAMP as a second messenger (activating PKA) are involved, the cGMP and PKG pathway is the most important. As mentioned above, PKGI-deficient mice showed deficient NO/cGMP-dependent relaxation of vascular smooth muscle. These mice have a very low ability to reproduce and relaxant responses to neuronally or endothelially released or exogenously administered NO in corpus cavernosum tissue were markedly reduced (Hedlund *et al.,* 2000). The activation of PKG and PKA initiates a series of cellular events *via* phosphorylation of various targets in the smooth muscle cell: inhibition of the noradrenergic pathway, opening of BK_{Ca} channels and activation of the Ca^{2+} ATPase transporter in the membrane of the sarcoplasmic reticulum. Eventually, these processes lead to a reduction in intracellular calcium levels and a consequent relaxation of arterial and trabecular smooth muscle. This results in vasodilatation and enhancement of blood flow into the cavernosal sinusoids and eventually penile erection. Blood becomes trapped in the corporal bodies by compression of subtunical venules against the tunica albuginea (full erection phase; Figure 2) and contraction of the voluntary ischiocavernosus muscle (rigid erection phase).

Since cGMP plays a key role in these processes, potential interventions for inadequate smooth muscle relaxation include increasing the level of intracellular cGMP. This can be achieved by PDE5 inhibition, but in the absence of stimulation of the NO pathway, PDE5 inhibition is ineffective. In human cavernosal tissue, at least 13 isoenzymes have been identified, including PDE3 (cGMP-inhibited cAMP PDE), PDE4 (cAMP-specific PDE) and PDE5 (cGMP-specific PDE). Functionally, PDE 3A and 5A seem to be the most important. Three of the PDE families (PDEs 5, 6 and 9) have a 100-fold substrate preference for cGMP over cAMP as substrate and are, therefore, considered to be cGMP-specific PDEs. PDE5 and PDE9 are the only cGMP-specific PDEs that are expressed in peripheral tissues; PDE6 is expressed in the retina (Lin, 2006; Francis *et al.,* 2011).

Clinical efficacy. Sildenafil citrate was the first effective oral treatment for ED, and its introduction marked a milestone in the ED history (Goldstein *et al.,* 1998; Osterloh, 2004). Current guidelines recommend PDE5 inhibitors as first-line therapy for most men with ED who do not have a specific contraindication to their use; they state that PDE5 inhibitors are effective, safe and well-tolerated therapies, and that there are no significant differences in efficacy, safety and tolerability between the approved drugs (Hatzimouratidis *et al.,* 2016). These recommendations are based on numerous trials, which have established both the efficacy and the safety profile. Sildenafil was the first (in 1998) compound introduced clinically followed by vardenafil and tadalafil (in 2003) and **avanafil** (in 2013). These drugs are available in most countries of the world. Other PDE5 inhibitors such as udenafil, mirodenafil and lodenafil are also available in some countries (Hatzimouratidis *et al.,* 2016).

The structural differences between these PDE5 inhibitors are small, but they have different pharmacokinetic (Table 1) and dynamic properties (Table 2). In clinical practice, this is reflected in their duration of action. The extended plasma half-time of tadalafil provides a longer therapeutic effect (up to 36 h), and this also might be the case for udenafil (up to 12 h). The duration of action for avanafil is longer than 6 h, that for sildenafil is at least 12 h and that for vardenafil is at least 10 h. The limited published data have shown that the onset and duration of action for mirodenafil and lodenafil are similar to those for sildenafil.

Overall, efficacy for the different PDE5 inhibitors rates is 60–70% with on-demand treatment regimens (Albersen *et al.*, 2010). Of the patients that initially do not respond to PDE5 inhibitors, between 30 and 50% may be converted to responders by counselling the patient and his partner. Some patients who fail to achieve an erection when taking PDE5 inhibitors on-demand benefit from a daily dosing regimen. Furthermore, in the male suffering from ED in the context of late onset hypogonadism, addition of testosterone supplementation might enhance PDE5 inhibitor therapy.

As the efficacy of PDE5 inhibitors depends on the integrity of the NO pathway in producing cGMP, it is evident that patients in whom this pathway is disturbed or defective will benefit far less than the general population from PDE5 inhibitors. Disease states that diminish NO availability include denervation of the erectile tissue following radical prostatectomy; severe diabetes with neuropathy and endothelial dysfunction; metabolic syndrome; and down-regulation of NOS expression as may be seen in atherosclerosis, ageing and hypogonadism.

Table 1

Pharmacokinetics of PDE5 inhibitors (from Hatzimouratidis *et al.*, 2016)

PDE5 inhibitor	T_{max} (h)	$t_{1/2}$ (h)	C_{max} (ng·mL^{-1})	AUC (ng × h·mL^{-1})
Avanafil 200 mg	0.75	5.1	2920	8490
Lodenafil 160 mg	1.2	2.4	157	530
Mirodenafil 100 mg	1.4	2.5	2989	7907
Sildenafil 100 mg	0.95	3.98	514	1670
Tadalafil 20 mg	2	17.5	378	8066
Vardenafil FCT 20 mg	0.66	3.9	20.9	74.5
Vardenafil ODT 10 mg	1.5	4.23	7.34	30.39
Udenafil 200 mg	0.76	9.88	1137	7898

AUC, area under the curve; C_{max}, maximum plasma concentration; FCT, film-coated tablet; ODT, oro-dispersible tablet; PDE5, phosphodiesterase type 5; $t_{1/2}$, time required for elimination of one half of the inhibitor from plasma; T_{max}, time required for attaining maximum plasma concentration. PK data were obtained after single-dose oral administration of the different PDE5 inhibitors.

Table 2

Pharmacodynamics of PDE5 inhibitors (modified from Hatzimouratidis *et al.*, 2016)

PDE5 inhibitor	MW g·mol^{-1}	MF	IC_{50} for PDE5 (nmol·L^{-1})	PDE selectivity
Avanafil	483.957	$C_{23}H26ClN_7O_3$	5.2	Highly selective for PDE5
Lodenafil	1035.206	$C_{47}H_{62}N_{12}O_{11}S_2$	0.015	Low activity against PDE1 and PDE6
Mirodenafil	531.672	$C_{26}H_{37}N_5O_5S$	0.33	Comparable to sildenafil for PDE5
Sildenafil	474.580	$C_{22}H_{30}N_6O_4S$	3.7	Low activity against PDE6 Very low activity against PDE1
Tadalafil	389.411	$C_{22}H_{19}N_3O_4$	1.8	Low activity against PDE11 Very low activity against PDE6
Vardenafil	408.607	$C_{23}H_{32}N_6O_4S$	0.091	Low activity against PDE6 Very low activity against PDE1
Udenafil	516.661	$C_{25}H_{36}N_6O_4S$	8.25	Comparable to sildenafil for PDE5

MF, molecular formula; IC_{50}, concentration that inhibits the effect by 50%.
The following sources/species were used for the determination of IC_{50} values: avanafil: canine lung (Kotera *et al.*, 2012); lodenafil: human platelets (Toque *et al.*, 2008); mirodenafil: source of PDE5 not indicated (Shin *et al.*, 2006); sildenafil, tadalafil and vardenafil: bovine PDE (Blount *et al.*, 2004); udenafil: human platelets (Doh *et al.*, 2002).

Adverse effects. The safety profile of the currently available PDE5 inhibitors is excellent, based on post-marketing data and further demonstrated by the recent FDA approvals for daily use of PDE5 inhibitors. These drugs are contraindicated in patients with unstable angina pectoris, recent myocardial infarction, certain arrhythmias and poorly controlled hypertension. Furthermore, patients who are treated with nitrates or nitrate-donors should not take PDE5 inhibitors, and use of PDE5 inhibitors with α_1-adrenoceptor antagonists may result in postural hypotension. The most common adverse events from PDE5 inhibitors are attributable to specific inhibition of PDE5 and vasodilatation in tissues other than the penis and include headache, facial and ocular hyperaemia, nasal congestion, myalgia and back pain. Adverse events account for about 25% of cases in which PDE5 inhibitors are discontinued, but the most common reason for discontinuation of PDE5 inhibitors being lack of efficacy. There have been rare reports of serious adverse events such as seizures, non-arteritic ischaemic optic neuritis and acute hearing loss. Some adverse events can be attributed to cross-reactivity with other PDE-isoforms (Table 3). Vision disturbances, which are believed to result from cross-reactivity of PDE5 inhibitors with PDE6 (an isoform of PDE that is abundantly present in the cones of the retina), have been reported with PDE5 inhibitor use. Tadalafil has been shown to cross-react with PDE11 to some extent, although no consequences of this cross-reactivity are currently known. None of the PDE5 inhibitors available has shown clinically significant cross-reactivity with PDE isoforms other than PDE6.

Symptoms associated with benign prostatic obstruction

Many epidemiological studies in different geographical areas have provided strong evidence that the association between LUTS and ED is not dependent on age. Several biological mechanisms have been implicated in this association, for example, the NO/cGMP pathway, **Rho-kinase**-mediated activation, autonomic hyperactivity, pelvic ischaemia/microvascular dysfunction, inflammatory pathways, sex hormones and psychological factors, but further research is required to better understand the molecular pathways involved. Evaluation of the possible impact of metabolic syndrome seems relevant (Cellek *et al.*, 2014; De Nunzio *et al.*, 2017).

PDE5 inhibitors cause relaxation of the bladder detrusor smooth muscle, and this effect is partly independent of NO (Oger *et al.*, 2010). Fusco *et al*. (2012) found that **hydrogen sulfide** (H_2S), which is predominantly formed from L-cysteine by the enzymes **cystathionine-β-synthase (CBS)** and **cystathionine-γ-lyase (CSE)**, may be involved in the relaxant effect of sildenafil in the human bladder. CBS and CSE were found to be present in the bladder dome and efficiently convert L-cysteine into H_2S, and both NaHS and L-cysteine relaxed human bladder strips. Sildenafil also caused relaxation of bladder strips and a concentration-dependent increase in H_2S production, and the authors suggested that this effect may account in part for the efficacy of PDE5 inhibitors in LUTS. Whether this opens up new therapeutic approaches remains to be established.

Rationale for use of PDE5 inhibitors. It is well documented that PDE5 inhibitors improve male LUTS, regardless of whether these symptoms are associated with ED (Uckert and Stief, 2011). The effects forming the rationale for use of PDE5 inhibitors in male LUTS may include improvement of LUT oxygenation, relaxation of smooth muscle, negative regulation of proliferation and trans-differentiation of prostate stroma, reduction of bladder afferent nerve activity and down-regulation of prostate inflammation (Andersson *et al.*, 2011; Giuliano *et al.*, 2013).

As mentioned above, PDE5 is expressed and has biological activity in all parts of the genitourinary tract (Figure 3) including the vasculature and urethral striated muscle (Lin *et al.*, 2006); however, with regard to its role in LUTS pathophysiology, focus has been on smooth muscle in the prostate, bladder and urethra (Fibbi *et al.*, 2010; Andersson *et al.*, 2011; Gacci *et al.*, 2016). Pathophysiological changes underlying the development of BPH-LUTS are multifaceted, and structural and functional changes in the bladder, prostate, supporting vasculature and innervation can affect bladder function (Andersson *et al.*, 2011; Gacci *et al.*, 2016).

Nomiya *et al*. (2013) found, in a rat model of chronic pelvic ischaemia, that treatment with tadalafil reduced bladder overactivity, decreased indicators of bladder ischaemia, normalized changes in NOS activity and prevented the accumulation of collagen. Thus, there might be several mechanisms generating afferent activity in the LUT, and changes in afferent signalling from the smooth muscle in the prostate, bladder and urethra seem to be the common mechanism in the onset of LUTS. This afferent activity is inhibited by PDE5 inhibitors. Interestingly, the mucosal effects of PDE5 inhibitors do not seem to have been specifically investigated.

Clinical efficacy. To date, only tadalafil has been approved for the treatment of LUTS secondary to BPH. The first clinical trial was conducted by Sairam *et al*. (2002) using

Table 3

Selectivity of the four worldwide available PDE5 inhibitors (Hatzimouratidis *et al.*, 2016)

Selectivity versus PDE5 (fold difference)				
PDE5 isoenzyme	**Avanafil**	**Sildenafil**	**Vardenafil**	**Tadalafil**
PDE1	>10 192	375	1012	10 500
PDE2	9808	39 375	273 810	>25 000
PDE3	>19 231	16 250	26 190	>25 000
PDE4	1096	3125	14 286	14 750
PDE5	1	1	1	1
PDE6	121	16	21	550
PDE7	5192	13 750	17 857	>25 000
PDE8	2308	>62 500	1 000 000	>25 000
PDE9	>19 231	2250	16 667	>25 000
PDE10	1192	3375	17 857	8750
PDE11	>19 231	4875	5952	25

sildenafil for treatment of LUTS/BPH/ED patients. After 3 months of treatment, there was a significant inverse relationship between international prostate symptom score (IPSS) and international index of erectile function score suggesting that sildenafil improved both LUTS and ED. Since then, the effects of PDE5 inhibitors, especially tadalafil, on LUTS/BPH have been extensively investigated. Many randomized controlled clinical trials (RCTs) have shown significant improvement in urinary symptoms and that the drug is well tolerated (Stief *et al.,* 2008; Roehrborn *et al.,* 2010; Martínez-Salamanca *et al.,* 2011; Gacci *et al.,* 2016). Several other PDE5 inhibitors may improve male LUTS; however, only tadalafil (5 mg once daily) has been licensed for the treatment of LUTS with or without ED.

The majority of the studies demonstrated that PDE5 inhibitors alone were efficacious in decreasing IPSS total score, storage subscore and voiding subscore, with the exception of the maximum urinary flow rate (Qmax). Oelke *et al*. (2014) showed that LUTS/BPH patients treated daily with tadalafil (5 mg) had greater treatment satisfaction compared with daily **tamsulosin** (0.4 mg) or placebo. Gacci *et al*. (2012) conducted an extensive pair-wise meta-analysis on the use of PDE5 inhibitors alone or in combination with α_1-adrenoceptor antagonists for the treatment of LUTS/BPH. They found that the combination of PDE5 inhibitors with α-adrenoceptor antagonists can significantly improve both LUTS and erectile function in men with BPH. This was confirmed by Wang *et al*. (2014) who found in a systematic review and network meta-analysis including 64 RCTs with 28 196 participants that combination of α_1-adrenoceptor antagonists together with PDE5 inhibitors was the most effective therapy.

Adverse effects. Liu *et al*. (2011) found that the relative risk of adverse events from tadalafil, vardenafil and sildenafil was 2.27, 1.86 and 1.22 respectively: the overall incidence of adverse events was 37.31% for PDE5 inhibitors compared with 24.03% for placebo, while serious adverse events were reported in 1.10% of men treated with tadalafil, 1.85% of those treated with vardenafil and 1.05% of those treated with sildenafil. The first meta-analysis of adverse events due to PDE5 inhibitors reported that flushing, gastro-oesophageal reflux, headache and dyspepsia were the most common side effects (odds ratio for occurrence: 4.88; 2.21; 1.88; 1.85; respectively). Moreover, regarding the overall tolerability of the association between PDE5 inhibitors and α_1-adrenoceptor antagonists, Gacci *et al*. (2012) described seven out of 103 adverse events (6.8%) with combined therapy and five of 99 (5.1%) in men treated with α-adrenoceptor antagonists alone. Similarly, flushing (4.37%), headache (4.23%), dyspepsia (3.69%), nasopharyngitis (2.27%) and dizziness (1.69%) were the most common treatment-related adverse events in a comparison network meta-analytic study on PDE5 inhibitors (Wang *et al.,* 2014).

Pulmonary arterial hypertension

PAH is defined as a group of diseases of the small pulmonary arteries, characterized by vascular narrowing leading to a progressive increase in pulmonary vascular resistance and increased right ventricle afterload. The consequence of this is failure of the afterload-intolerant right ventricle, eventually leading to premature death (Hampl and Herget, 2000; Humbert *et al.,* 2004). PAH has a multifactorial pathobiology and pathophysiological mechanisms of the disease include pulmonary endothelial dysfunction, which leads to impaired production of vasodilators, such as NO and prostacyclin, and overexpression of vasoconstrictors, such as endothelin-1 (Figure 4). However, vascular proliferation and remodelling of the bladder wall is the hallmark of the pathogenesis. The multifactorial pathophysiology implies that treatment needs to be individualized and that one therapy may not be optimal for all patients. This is reflected in the number of drugs used for treatment. At present, there are five approved classes of drugs: **endothelin receptor** antagonists, PDE5 inhibitors, prostacyclin (PGI_2) analogues, calcium channel blockers and sGC stimulators (Desai and Desouza, 2017) (Figure 4). Combination therapy may be useful, but therapy should be individualized.

Rationale for use of PDE5 inhibitors. Corbin *et al*. (2005) demonstrated a high level of PDE5 in lung, approximately as high as that in penile corpus cavernosum, and suggested that the abundance of PDE5 in lung vascular smooth muscle may provide a strong molecular basis for PDE5 inhibitor treatment of PAH. It has been shown that PDE5 is up-regulated in conditions associated with PAH, and by selectively inhibiting PDE5 with, for example, sildenafil citrate, NO-mediated vasodilatation in the lung can be obtained; PDE5 inhibition may also have antiproliferative effects on pulmonary vascular smooth-muscle cells. However, the efficacy of PDE5 inhibitors is dependent on the presence of sufficient amounts of endogenous NO to activate sGC and generate cGMP. Since NO dilates smooth muscle cells by increasing intracellular cGMP levels, low endogenous NO/cGMP production significantly limits or impairs the effects of PDE5 inhibitors. Inhaled NO is the first-line vasodilator therapy in persistent pulmonary hypertension of the newborn and is commonly used in the intensive care unit (Kim *et al.,* 2016). In term and near-term infants with hypoxia, oxygenation was improved in approximately 50% of infants receiving inhaled NO (Barrington *et al.,* 2017). PDE5 inhibitor therapy is the most commonly recommended oral treatment option in children with PAH (Kim *et al.,* 2016). For PDE5 inhibitors to be effective, there must be sufficient activity of the NO/sGC/cGMP pathway. If there is insufficient NO production to stimulate sGC, an alternative way is the use of NO-independent sCG stimulators (e.g. riociguat: BAY 63–2521). PGI_2 (prostacyclin) induces relaxation of vascular smooth muscle by stimulating the production of cAMP and inhibits the growth of smooth-muscle cells. In addition, it is a powerful inhibitor of platelet aggregation. Inhibitors of PDE3, such as **milrinone**, stabilize the cAMP concentrations, but milrinone is not commonly used in the treatment of PAH.

Clinical efficacy. Galiè *et al*. (2005) performed a double-blind, placebo-controlled study of the effects of placebo or sildenafil (20, 40 or 80 mg, p.o.) three times a day for 12 weeks in 278 randomly assigned patients with symptomatic PAH. The primary measure of efficacy was the change in exercise capacity, as measured by the total distance walked in 6 min, from baseline to week 12. They

found that sildenafil improved exercise capacity, WHO functional class and haemodynamics. Most adverse events were mild to moderate in intensity for all treatment groups. Unegbu *et al*. (2017) performed a systematic review of the comparative effectiveness and safety of PDE5 inhibitors in the management of paediatric patients with PAH. They found strong evidence that PDE5 inhibitor use improves echocardiography measurements, cardiac catheterization parameters and oxygenation compared with baseline or placebo. There is also evidence that low- and moderate-doses of sildenafil are safe regimens for children. In a meta-analysis assessing the effects of PDE5 inhibitors in patients suffering from PAH due to left chronic heart failure, De Vecchis *et al*. (2017) found that among patients (>18 years old) with reduced left ventricular ejection fraction, a significant benefit was conferred by PDE5 inhibitors against the risk of the composite endpoint of death and hospitalization. In contrast, patients with preserved left ventricular ejection fraction had no benefit from PDE5 inhibitor treatment.

Adverse effects. The safety profile has generally been benign with headaches, flushing, dyspepsia, diarrhoea, nasal congestion and tinnitus among the most commonly reported side effects (Galiè *et al.,* 2005; Unegbu *et al.,* 2017).

Conclusions

The NO/cGMP pathway is involved in many normal physiological functions and in the pathophysiology of a wide range of diseases. PDE5 inhibitors have not only been invaluable tools for the study of the functions and dysfunctions of the NO/cGMP pathway but have also been used as therapeutic agents as a result of their effects on, for example, smooth muscle, cardiovascular tissues and platelets. Since the discovery and introduction of sildenafil, inhibitors of PDE5 have been the first-line therapy for ED, and the success of sildenafil has stimulated research in the field and led to a number of new applications, such as treatment of LUTS and PAH, now approved indications. PDE5 isoenzymes have been identified in a wide variety of tissues, which should imply that PDE5 inhibitors will have an effect on many organs and functions, and they have also been used for many not yet approved indications, such as diabetes and cancer, and the fields of clinical use are increasing (Gur *et al.,* 2012; Ribaudo *et al.,* 2016). Even though PDE5 inhibitors are useful treatments of many diseases, they are by no means a panacea, and new clinical applications should always be guided by the results of randomized clinical trials.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.,* 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.,* 2017a,b,c).

Conflict of interest

The author declares no conflicts of interest.

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