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Review

Phosphodiesterase 5 inhibitors and nitrergic transmission—from zaprinast to sildenafil

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Abstract

Phosphodiesterase 5 terminates the cellular actions of the second messenger molecule cyclic GMP; inhibitors of phosphodiesterase 5 will therefore increase and prolong the actions of endogenous substances that signal via the cyclic GMP pathway, including nitric oxide released as a neurotransmitter from nitrergic nerves. To date, the most widely used phosphodiesterase 5 inhibitors, zaprinast and sildenafil, have proved vital in the elucidation of the widespread role of cyclic GMP in nitrergic transmission and, specifically in the case of sildenafil, have provided a major breakthrough in the treatment of erectile dysfunction in men. Although still a matter of debate, early evidence indicates that sildenafil may also be of benefit in some forms of sexual dysfunction in women. The remarkable clinical success of sildenafil has prompted the search for further novel phosphodiesterase 5 inhibitors which might be used to enhance nitrergic function in other disease states.

Keywords: cGMP; Erectile dysfunction, male; Nitrergic nerve; Nitric oxide (NO); Phosphodiesterase 5; Sexual dysfunction, female; Sildenafil; Zaprinast

1. Introduction

The guanylyl cyclase/cyclic GMP cell signalling system was identified soon after its sister nucleotide system adenylyl cyclase/cyclic AMP (for reviews see Waldman and Murad, 1987; Goy, 1991; Hobbs, 1997; Wedel and Garbers, 1998). However, while advances in our understanding of the cellular functions of cyclic AMP followed on quickly from its discovery, the same was not true for cyclic GMP. The main reasons for this delay were the lower (about 10 times) concentrations of cyclic GMP in cells compared with cyclic AMP, the diverse forms (particulate and soluble) of the cyclic GMP synthesising enzyme guanylyl cyclase, and the problem of identifying the endogenous substances utilising the guanylyl cyclase/cyclic GMP pathway as a cellular signal. During the 1980s, however, it became clear that the multiple particulate forms of guanylyl cyclase were the receptors for a variety of endogenous peptides (for example, the atrial natriuretic peptides and guanylin), while soluble guanylyl cyclase was the target for nitric oxide released from either vascular endothelium (endothelium-derived relaxing factor) or from certain types of non-adrenergic, non-cholinergic nerves (nitrergic transmitter). In parallel with the elucidation of the pathways which generate cyclic GMP, the structure and function of the phosphodiesterase enzymes responsible for inactivating the cyclic nucleotide were being identified (Thompson, 1991). In terms of the nitric oxide/soluble guanylyl cyclase/cyclic GMP pathway, drugs were developed which could either inhibit (nitric oxide synthase inhibitors) or potentiate (phosphodiesterase inhibitors) this novel cell signalling system; of these drugs, selective inhibitors of the cyclic GMP-specific phosphodiesterase (phosphodiesterase 5) have provided the most exciting and dramatic advance in therapeutics. The purpose of the present review is to discuss the interaction of such phosphodiesterase 5 inhibitors with the autonomic component of the nitrergic nervous system, concentrating on the two drugs which have been most widely used—zaprinast and sildenafil. The signalling path-

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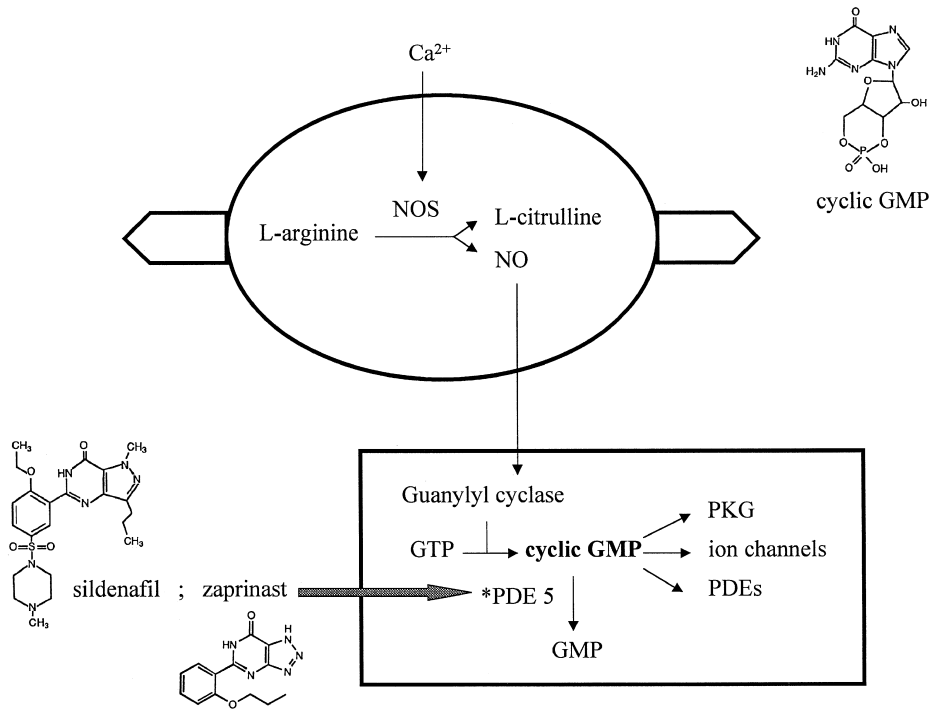

Fig. 1. Diagram representing the nitrergic neuroeffector junction. Influx of Ca^{2+} during depolarisation activates nitric oxide synthase (NOS) in the nerve terminal. The nitric oxide (NO) so produced diffuses from the nerve to the postjunctional cell and activates guanylyl cyclase to generate cyclic GMP. This second messenger molecule regulates cell activity by interacting with various targets, including protein kinase G (PKG), cyclic nucleotide gated ion channels, and cyclic GMP-regulated phosphodiesterases (PDEs). The actions of cyclic GMP are terminated by its metabolism to GMP by phosphodiesterase 5. Zaprinast and sildenafil are selective inhibitors of phosphodiesterase 5 (* PDE 5); they have structures related to cyclic GMP and compete with the nucleotide for binding to the catalytic site on the enzyme. In this way, the inhibitors increase and prolong the effects of nitrergic nerve stimulation.

way involved and the structures of the phosphodiesterase 5 inhibitors in relation to cyclic GMP are shown in Fig. 1.

2. Cyclic GMP-specific phosphodiesterase 5

The superfamily of mammalian phosphodiesterase enzymes comprises at least 11 individual members, each derived from separate genes (Thompson, 1991; Corbin and Francis, 1999; also Fawcett et al., 2000). The enzymes vary in their substrate specificity; some are non-selective between cyclic AMP and cyclic GMP (phosphodiesterases 1, 2, 3, 10, 11), while others are selective for either cyclic AMP (phosphodiesterases 4, 7, 8) or cyclic GMP (phosphodiesterases 5, 6, 9). The phosphodiesterase enzymes cleave the cyclic nucleotide phosphodiester bond at the 3′-position by an action involving the Zn^{2+} binding motifs located in the catalytic domain of the enzyme; they therefore resemble Zn^{2+}-metalloendoproteases in this respect (Corbin and Francis, 1999).

Cyclic GMP-specific phosphodiesterase 5 was first identified in lung (Lincoln et al., 1976) and was subsequently purified and cloned (Francis et al., 1980; Francis and Corbin, 1988; Thomas et al., 1990; McAllister-Lucas et al., 1993). The protein is abundant in smooth muscle and is considered to be the main enzyme responsible for terminating the action of cyclic GMP generated following the release of nitric oxide from nitrergic nerves (or, of course, from vascular endothelial cells); drugs which inhibit phosphodiesterase 5 should therefore enhance and prolong the effects of nitrergic stimulation.

Phosphodiesterase 5 is a homodimer, each subunit being composed of 875 amino acids. However, recent studies with a mutant phosphodiesterase 5 consisting only of the monomeric 357 amino acid catalytic domain showed that this fragment retained the essential catalytic features of the full-length enzyme and therefore that catalytic activity does not require either dimerisation or interaction between the regulatory and catalytic domains (Fink et al., 1999). The proposed components of the phosphodiesterase 5 subunits are represented in Fig. 2.

The amino-terminal regulatory domain of phosphodiesterase 5 contains a phosphorylation site (Ser^{92}) which can be phosphorylated by either protein kinase A or protein kinase G; such phosphorylation results in a 50–70% increase in enzyme activity (Corbin et al., 2000). Each of the sub-units also contains two allosteric (non-catalytic) binding sites for cyclic GMP and occupation of these sites is necessary for phosphorylation of Ser^{92}; further, binding of cyclic GMP to the allosteric sites is enhanced following occupation of the catalytic sites (Corbin and Francis, 1999). The catalytic domain (amino acids 578–812) is located at the carboxy terminus and contains a cyclic GMP binding domain and a Zn^{2+} binding motif, mentioned above,

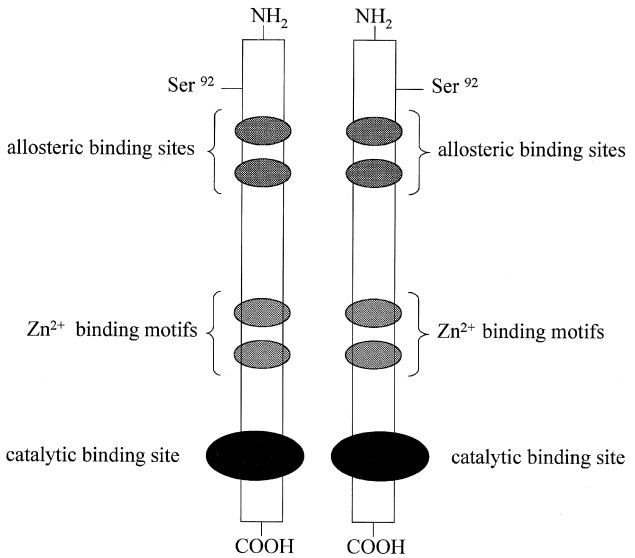

Fig. 2. Diagram of the main molecular features of phosphodiesterase 5. The enzyme is a homodimer with each subunit having 875 amino acids. In addition, each subunit has a phosphorylation site (Ser^{92}), two allosteric binding sites for cyclic GMP, two Zn^{2+}-binding motifs and a catalytic binding site. The importance of these sites, and their interactions, are explained in the text.

which overlaps the catalytic site; the Zn^{2+} may be involved in the production of OH^- which attacks the phosphodiester bond of the nucleotide substrate (Francis et al., 1994). Site-directed mutagenesis studies support an important role for amino acid residues His^{603}, His^{607}, His^{643}, His^{647}, Glu^{672}, Asp^{714} and Asp^{754} in catalysis (Turko et al., 1998). Overall, the proposed sequence of enzyme activation and regulation is that elevated levels of cyclic GMP in the cytoplasm result in enhanced binding of the nucleotide to the catalytic site; this, in turn, promotes binding of cyclic GMP to the allosteric sites, allowing phosphorylation of Ser^{92} and increased enzyme activity (Corbin and Francis, 1999). This provides a highly regulated mechanism by which the cell can control cytoplasmic concentrations of cyclic GMP.

Phosphodiesterase 5 inhibitors, such as zaprinast and sildenafil, compete with cyclic GMP for binding to the catalytic site but not to the allosteric sites (Corbin and Francis, 1999). Like cyclic GMP itself, occupation of the catalytic sites by the phosphodiesterase 5 inhibitors stimulates binding of cyclic GMP to the allosteric sites; however, phosphorylation of Ser^{92} does not alter the binding of the phosphodiesterase 5 inhibitors to the catalytic domain (Corbin et al., 2000). Site-directed mutagenesis suggests that the amino acids critical for binding of the inhibitors within the catalytic domain do not completely match those responsible for nucleotide binding. Indeed, recent evidence shows that the binding patterns of sildenafil and zaprinast also differ within the catalytic domain (Turko et al., 1999). The binding of sildenafil is more closely allied to that of cyclic GMP, which may explain the much greater potency of sildenafil over zaprinast as a phosphodiesterase 5 inhibitor (sildenafil IC_{50} 1 nM; zaprinast IC_{50} 130 nM). The amino acids identified as important for sildenafil binding (Tyr^{602}, His^{607}, His^{643}, Asp^{754}) are found in all forms of mammalian phosphodiesterases and so the selectivity of the drug for phosphodiesterase 5 may be due to some other unique aspect of the protein (Turko et al., 1999); recently, three distinct isoforms of phosphodiesterase 5 have been identified in human corpus cavernosum (Lin et al., 2000) and so it is possible that even more selective inhibitors may be discovered.

3. Zaprinast

Zaprinast (M & B 22948; 2-*O*-propoxyphenyl-8-azapurin-6-one) was synthesised during a study of xanthines as possible anti-allergy compounds (Broughton et al., 1974). It was already known that xanthines such as theophylline could inhibit the release of inflammatory mediators from leukocytes and mast cells, giving them some anti-allergy potential, but they were some 20–50 times less potent than disodium cromoglycate. Zaprinast, however, was found to be 20–50 times more potent than disodium cromoglycate as an inhibitor of histamine release, asthma-induced bronchospasm, and the spasmogenic action of histamine and other agents on smooth muscle. The drug was therefore proposed as a possible candidate for use in allergic asthma. Subsequent studies showed that zaprinast, like other xanthines, could inhibit phosphodiesterases and that it was a selective inhibitor of cyclic GMP-specific phosphodiesterase in human lung, rat mast cells and bovine coronary artery (some 200 times more potent against cyclic GMP phosphodiesterase than cyclic AMP phosphodiesterase); in addition, zaprinast greatly potentiated the relaxant effects of nitrovasodilator drugs in the coronary artery (Bergstrand et al., 1977; Bergstrand et al., 1978; Kukovetz et al., 1979).

The ability of zaprinast to potentiate non-adrenergic, non-cholinergic relaxations of smooth muscle was demonstrated by Bowman and Drummond (1984). Based on results showing that haemoglobin inhibits non-adrenergic, non-cholinergic relaxations of the bovine retractor penis, they investigated the hypothesis that the as yet unidentified non-adrenergic, non-cholinergic transmitter may produce smooth muscle relaxation via cyclic GMP production. This proved to be the case since (1) non-adrenergic, non-cholinergic stimulation resulted in elevated cyclic GMP levels in the smooth muscle, (2) haemoglobin and *N*-methyl-hydroxylamine (a weak guanylyl cyclase inhibitor) prevented this rise in cyclic GMP and the associated relaxation, and (3) zaprinast enhanced both effects of non-adrenergic, non-cholinergic stimulation. Subsequently, zaprinast was shown to increase relaxations of the mouse anococcygeus induced by non-adrenergic, non-cholinergic

nerve stimulation and nitric oxide donors, but to have no effect on relaxations to vasoactive intestinal peptide, papaverine or 3-isobutyl-1-methylxanthine (Gibson and Mirzazadeh, 1989). These results with the retractor penis and anococcygeus strongly suggested that the non-adrenergic, non-cholinergic transmitter of these two tissues utilised the guanylyl cyclase/cyclic GMP signalling pathway, and that the transmitter had much in common with nitric oxide donor drugs. Indeed, shortly after this, experiments with nitric oxide synthase inhibitors established that the non-adrenergic, non-cholinergic transmitter in these and certain other tissues was in fact nitric oxide itself, and the neurotransmission system was termed 'nitrergic' (Rand, 1992; Rand and Li 1995a,b). While the nitric oxide synthase inhibitors proved crucial in the identification of nitrergic transmission, zaprinast was widely used to establish the important role of cyclic GMP as the second messenger generated in the smooth muscle by nitric oxide. Table 1 lists a range of tissues in which nitrergic responses were shown to be increased in amplitude and/or duration in the presence of zaprinast. Of particular relevance to the subsequent introduction of sildenafil into the clinic were the observations that zaprinast enhanced nitrergic relaxations of the corpus cavernosum and facilitated penile erection in the dog (Table 1). The drug has also been shown to inhibit contractile responses to adrenergic nerve stimulation in vascular tissue, although this effect is probably due to enhancement of the relaxant actions of endothelial nitric oxide opposing the nerve-induced contraction (Bucher et al., 1992; Tesfamariam et al., 1992).

Table 1
Examples of tissues/preparations in which zaprinast enhances nitrergic responses

Tissue/preparation	Species	Reference
bronchus	man	Fernandes et al., 1994
trachea	guinea pig	Ellis and Conanan, 1995
airways	cat	Imoto et al., 1998
gastric fundus	cat	Barbier and Lefebvre, 1995
gastric fundus	pig	Lefebvre et al., 1995
gastric fundus	rat	Williams and Parsons, 1995
pyloric sphincter	dog	Bayguinov and Sanders, 1993
ileum	hamster	Matsuyama et al., 1999
ileocolonic sphincter	dog	Ward et al., 1992a
proximal colon	dog	Ward et al., 1992b
proximal colon	dog	Shuttleworth et al., 1993
internal anal sphincter	guinea pig	Rae and Muir, 1996
coeliac plexus	rabbit	Quinson et al., 1999
urinary bladder	sheep	Thornbury et al., 1995
urethra	rabbit	Dokita et al., 1991
urethra	rabbit	Persson and Andersson, 1994
anococcygeus	rat	Mirzazadeh et al., 1991
anococcygeus	mouse	O'Kane and Gibson, 1999
corpus cavernosum	man	Rajfer et al., 1992
corpus cavernosum	man, rabbit	Bush et al., 1992
penile erection	dog	Trigorocha et al., 1993
clitoris	rabbit	Cellek and Moncada, 1998
mesenteric artery	bull	Ahlner et al., 1991
pulmonary artery	guinea pig	Liu et al., 1992
pulmonary vascular bed	cat	McMahon et al., 1993

4. Sildenafil

Sidenafil citrate (Viagra™) was discovered during a rational drug design programme investigating 5-(2′-alkoxyphenyl)pyrazol[4,3-*d*]pyrimidin-7-ones as selective inhibitors of phosphodiesterase 5 (Terret et al., 1996). Originally placed under clinical trial for the treatment of cardiac conditions it became clear that the drug might have greater therapeutic potential for the treatment of erectile dysfunction in men; this proved to be the case and sildenafil became licensed for clinical use in 1998. In parallel with these clinical observations with sildenafil, the role of the nitric oxide/guanylyl cyclase/cyclic GMP signalling pathway in penile erection was becoming apparent (Naylor, 1998), thereby providing the scientific basis for the clinical observations with the drug. In this review, the in vitro results with sildenafil in nitrergically innervated tissues will be discussed first, followed by a brief overview of the clinical experience with the drug to date.

4.1. In vitro and biochemical studies with sildenafil in male urogenital smooth muscle

Most in vitro studies with sildenafil have been carried out using the corpus cavernosum from various species (Jeremy et al., 1997; Ballard et al., 1998; Chuang et al., 1998; Moreland et al., 1998; Stief et al., 1998). In rabbit, sildenafil, either by itself or in combination with sodium nitroprusside, was found to increase cyclic GMP production in corpus cavernosum segments with an EC_{50} value of around 500 nM (Jeremy et al., 1997); tissue levels of cyclic AMP were unaffected. When phosphodiesterase 5 was extracted from human corpus cavernosum, it was inhibited in a competitive manner by sildenafil, with an IC_{50} of 4 nM (Ballard et al., 1998); this compared with IC_{50} values against phosphodiesterases 1, 2, 3, 4 and 6 of 281, $>30{,}000$, 16,200, 7680 and 33 nM, respectively. Sildenafil was some 240 times more potent than zaprinast (IC_{50}, 856 nM) as an inhibitor of the extracted phosphodiesterase 5 (Ballard et al., 1998). In functional studies, sildenafil (10–1000 nM) enhanced both the amplitude and duration of relaxations induced by field stimulation of the nitrergic nerves (Ballard et al., 1998). The effect on amplitude was greater at lower frequencies of field stimulation (1–2 Hz) with a maximum threefold increase in relaxation being observed with 1000 nM sildenafil; this same concentration increased the duration of nitrergic relaxations by twofold at 8 Hz. Interestingly, sildenafil had little effect on phenylephrine-induced tone in the absence of field stimulation. Moreland et al. (1998) also found that sildenafil produced competitive inhibition of phosphodiesterase 5

from human corpus cavernosum (IC_{50} 4–6 nM) and enhanced cyclic GMP generation in response to sodium nitroprusside. These results therefore showed that sildenafil potently potentiated relaxations mediated by nitrergic field stimulation of urogenital smooth muscle from males. Importantly, sildenafil itself produced very little direct relaxation of the muscles, suggesting that there was little 'tonic' release of nitric oxide under resting conditions; it was likely therefore that in vivo the potentiating effects of the drug would only be observed during periods of nitrergic nerve activity. Indeed, this proved to be the case. Pelvic nerve stimulation in the pentobarbitone-anaesthetised dog resulted in increased intracavernosal pressure and penile blood flow (Carter et al., 1998); the involvement of nitrergic nerves in these responses was confirmed by the inhibitory effect of a nitric oxide synthase inhibitor. Administration of sildenafil to the dogs by itself had no effect on penile erection, general blood pressure or heart rate. However, in the presence of sildenafil, the effects of pelvic nerve stimulation were potentiated.

Typical examples of the effects of sildenafil on nitrergic relaxations of the mouse anococcygeus are shown in Figs. 3 and 4 (Frith and Gibson, 2000). On the frequency–response curve using a fixed number of pulses, 50 nM sildenafil produced a clear potentiation of the relaxations (Fig. 3); as found in corpus cavernosum (Ballard et al., 1998), the increase in relaxation amplitude was greatest at low frequencies of stimulation, while the increase in duration was much greater at high frequencies. Fig. 4 shows that when the muscle was stimulated intermittently (5 Hz trains for 30 s every 30 s) the relaxations were pulsatile. However, in the presence of sildenafil the relaxation was not only enhanced but, because of the increased duration of each pulse, the overall relaxation became essentially sustained. This may be of relevance to the therapeutic application of sildenafil since there is evidence that parasympathetic nerves may fire intermittently under physiological conditions (Tobin et al., 1990) and, further, sildenafil is very effective in the treatment of erectile dysfunction in patients with diabetes and spinal cord injury (see later), in which the pattern of nerve firing will be compromised.

4.2. In vitro and biochemical studies with sildenafil in female urogenital smooth muscle

Nitrergic nerves also innervate smooth muscle structures of the female urogenital tract, including the clitoris (Burnett et al., 1997; Cellek and Moncada, 1998), and there has been growing interest in the possible use of phosphodiesterase 5 inhibitors in the treatment of female sexual dysfunction (see later). The basic scientific rationale for such clinical application has been confirmed by in vitro studies. In human clitoris, sildenafil inhibited phosphodiesterase 5 activity both in crude extracts (IC_{50}, 50 nM) and in partially purified enzyme preparations (IC_{50}, 7 nM); it also enhanced the effects of sodium nitroprusside (Park et al., 1998). In terms of functional nerve responses, sildenafil has been shown to increase both the amplitude and duration of nitrergic relaxations in rabbit clitoral corpus cavernosum (Vemulapalli and Kurowski, 2000). The relative potency of sildenafil in male and female urogenital

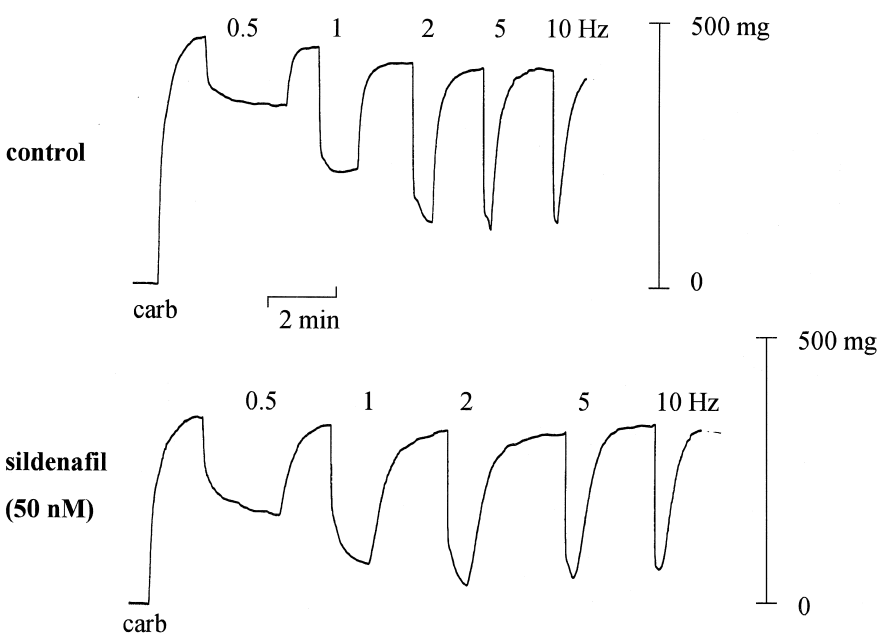

Fig. 3. Traces showing the effects of sildenafil on nitrergic relaxations of an anococcygeus muscle from a male mouse. The muscle was field-stimulated with trains of 100 pulses at varying frequencies (0.5–10 Hz) in the absence, and then in the presence, of 50 nM sildenafil. Tone was raised with 50 μM carbachol (carb). In the presence of sildenafil, nitrergic relaxations were increased both in amplitude (especially at low frequencies) and duration (especially at high frequencies).

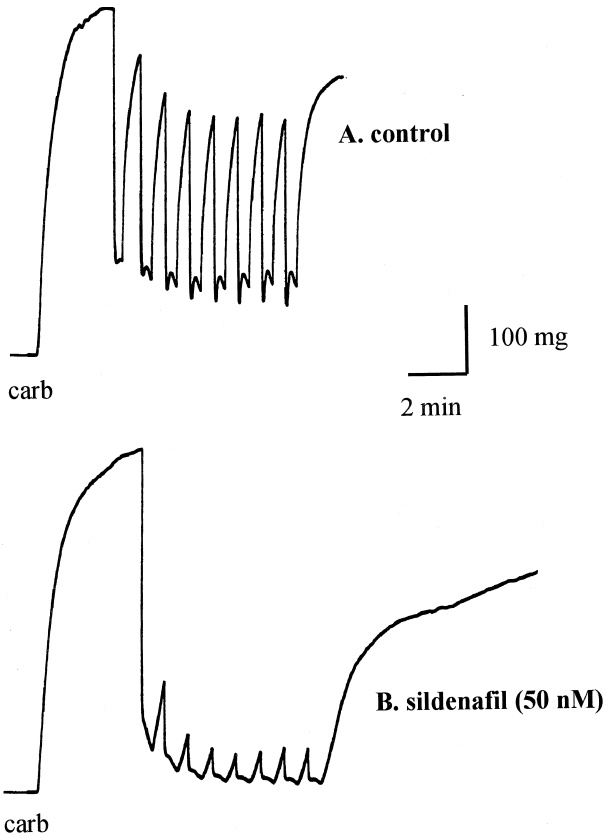

Fig. 4. Traces showing the effects of sildenafil on nitrergic relaxations of an anococcygeus muscle from a male mouse. In this case, the muscle was stimulated intermittently (5 Hz trains for 30 s every 30 s) in the absence, and then in the presence, of 50 nM sildenafil. Tone was raised with 50 μM carbachol (carb). In the absence of sildenafil, the relaxations were pulsatile; in its presence the relaxations were larger and became essentially sustained.

smooth muscle has been compared using the mouse anococcygeus (Frith and Gibson, 2000). In muscles from both sexes, sildenafil enhanced nitergic relaxations with an EC_{50} of around 30 nM in each case; the enhancement was seen both as an increase in response magnitude (especially at low frequencies of nitrergic stimulation) and response duration (dominant at higher frequencies). The selectivity of sildenafil was confirmed by the use of various relaxant drugs; relaxations to nitric oxide and sodium nitroprusside were enhanced in a manner similar to those to nitrergic nerve stimulation, while relaxations to papaverine and forskolin were unaffected. This latter result is of interest since it suggests that there is little or no 'cross-talk' between the cyclic GMP and cyclic AMP pathways in the anococcygeus, although sildenafil has recently been reported to increase cyclic AMP levels in human corpus cavernosum (Stief et al., 2000).

4.3. Clinical use of sildenafil in men

Erectile dysfunction is a widespread, age-related condition which has a major impact on the quality of life; the incidence of erectile dysfunction increases by two- to threefold (depending on the severity of disease) between the ages of 40 and 70, and it is estimated that about half the male population over 70 years of age suffers from some form of erectile dysfunction. The introduction of sildenafil citrate represents a major advance in the management of erectile dysfunction, being the first really effective oral therapy (Boolell et al., 1996). Since its introduction in 1998, there have been several reports of clinical studies confirming the effectiveness of sildenafil in the treatment of erectile dysfunction associated with a wide range of aetiologies (Goldstein et al., 1998; Truss and Stief, 1998; Padma-Nathan et al., 1998; Moreland et al., 1999; Steers 1999). As might be expected, the aetologies include those associated with damage to the nitrergic structures controlling erection, such as diabetes mellitis, spinal cord injury, and treatments for prostate cancer. It has been estimated that around 40% of men with erectile dysfunction also suffer from diabetes mellitis and there is good evidence that sildenafil is well tolerated and effective in such individuals (Price et al., 1998; Spollett, 1999; Cummings and Alexander, 1999). Similarly, there is a marked improvement in the quality of life of men suffering from erectile dysfunction as a result of spinal cord injury; the degree of improvement in erectile function is inversely related to the degree of nerve damage and in patients with a complete loss of sacral (S1–S4) activity drug treatment is ineffective (Derry et al., 1998; Maytom et al., 1999; Hultling et al., 2000; Schmid et al., 2000). Erectile dysfunction as a result of radical prostatectomy also responds well to sildenafil, although again the effectiveness of the treatment depends on the integrity of the nitrergic nerves. Thus, sildenafil produced a positive effect in 72% of patients who had undergone prostatectomy with bilateral nerve-sparing surgery, 50% in those with unilateral nerve-sparing surgery, and 15% in those receiving only non-nerve-sparing surgery (Zippe et al., 2000). The beneficial effects of sildenafil are not limited to the after-effects of prostate surgery; over two-thirds of patients receiving radiotherapy for the treatment of prostate cancer also showed significant enhancement of erectile function following sildenafil administration (Zefelesky et al., 1999).

In terms of its clinical activity, maximum plasma levels of sildenafil following oral administration occur after 60–120 min, with around 96% of the drug being bound to plasma proteins (Moreland et al., 1999; Steers, 1999); the plasma half-life of sildenafil and its active metabolite (via cytochrome P_{450}) is around 4–4.5 h. The main side effects, which are related to phosphodiesterase inhibition in other tissues, include headache, facial flushing, dyspepsia, rhinitis and visual disturbances. The drug is contra-indicated in patients receiving concurrent nitrovasodilator therapy, since the precipitative fall in blood pressure can be fatal. Sildenafil administration by itself, in the absence of sexual stimulation, does not cause penile erection, in agreement with the findings in animal tissues that the main effects of

the drug are only observed during nitrergic nerve activation.

4.4. Clinical use of sildenafil in women

As in men, sexual dysfunction in women is widespread and age-related; it is estimated that about 30–50% of the female population suffers from some kind of sexual dysfunction (Berman et al., 2000). The disease in women has been divided into four main categories:

1. hypoactive sexual desire disorder
2. sexual arousal disorder
3. orgasmic disorder
4. sexual pain disorder

As described earlier, there is good evidence that nitrergic nerves innervate, and cause relaxation of, smooth muscle structures within the female urogenital tract and that responses to these nerves are enhanced by phosphodiesterase 5 inhibitors, including sildenafil (Burnett et al., 1997; Cellek and Moncada, 1998; Park et al., 1998; Frith and Gibson, 2000; Vemulapalli and Kurowski, 2000). Consequently, there has been growing interest in the possibility that the phosphodiesterase 5 inhibitors may be of use in alleviating sexual dysfunction in women, particularly in terms of the treatment of some of the aspects of category 2 above (arousal disorder) such as vaginal lubrication and clitoral engorgement/sensitivity. To date, however, the results of clinical trials with sildenafil have been mixed and suggest that the effectiveness of the treatment may depend on the patient population.

In a study of 33 menopausal women with a history of sexual dysfunction, sildenafil (50 mg) was found to have no significant overall effect on sexual function (as measured subjectively by the patients). The drug was, however, well tolerated and did produce improvements in both vaginal lubrication and clitoral sensitivity (Kaplan et al., 1999). Conversely, a recent study of 19 premenopausal women with spinal cord injuries found that 50 mg sildenafil did result in significant improvements in sexual arousal and vaginal responses to sexual stimulation; side effects were minor with only slight changes in heart rate and blood pressure (Sipski et al., 2000). Sildenafil is also beneficial in the treatment of sexual dysfunction related to anti-depressant therapy, specifically with selective serotonin re-uptake inhibitors; the effects of sildenafil can be observed in both men and women undergoing antidepressant therapy (Shen et al., 1999; Nurnberg et al., 1999, 2000).

A possible further use for sildenafil may be to aid the chances of success in women undergoing in vitro fertilisation since vaginal application of sildenafil was found to increase blood flow through the uterus and, when combined with oestrogen, to enhance endometrial thickness (Sher and Fisch, 2000).

Thus, the results of early studies with sildenafil in women are encouraging. However, further studies observing a greater number, and wider range, of patients will be important to determine the types of sexual dysfunction/conditions which will respond positively to the drug.

5. Other phosphodiesterase 5 inhibitors

The discovery of the important and widespread role played by the nitrergic nervous system, together with the success of sildenafil as a therapeutic agent, has prompted the search for other selective inhibitors of phosphodiesterase 5. Compounds which have been investigated include 1-cyclopentyl-3-methyl-6-(4-pyridyl)pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (WIN-58237; Silver et al., 1994; K_i 170 nM against phosphodiesterase 5; but also 300 nM against phosphodiesterase 4, so of limited selectivity), sodium 1-[6-chloro-4-(3,4-methylenedioxybenzyl)-aminoquinazolin-2-yl]piperidine-4-carboxylcarboxylate sesquihydrate (E4021; Kodama et al., 1994; Adachi and Nishino, 1998; D'Amours et al., 1999; Ohnishi et al., 1999; K_i 1.7 nM similar against both phosphodiesterases 5 and 6), 1-cyclopentyl-3-ethyl-6-(3-ethoxypyrid-4-yl)-1*H*-pyrazolo-[3,4-*d*]pyrimidin-4-one (SR 265579; Kapui et al., 1999; K_i 6.4 nM against phosphodiesterase 5; 33 times and 14 times less potent as an inhibitor of phosphodiesterases 3 and 4, respectively), and 4-[2-(2-hydroxyethoxy)ethylamino]-2-(1*H*-imidazol-1-yl)-6-methoxy-quinazoline methanesulphonate (ONO-1505; Laight et al., 1999). There have also been preliminary reports of a sildenafil analogue with greater potency than the parent compound (Voelter et al., 1999). However, perhaps the most interesting compounds to date are the N-3-substituted imidazoquinazolinones, one of which (*N*-3-(fluorobenzyl)-imidazoquinazolinone) showed greater potency than sildenafil against phosphodiesterase 5 (K_i of 0.48 nM as opposed to 1.6 nM) and was more selective, even against phosphodiesterase 6; however, the drug was equi-effective with sildenafil in terms of potentiating nitrergic relaxations of the rabbit corpus cavernosum (Rotella et al., 2000).

6. Conclusions and future directions

Selective inhibitors of phosphodiesterase 5 have made major contributions to our understanding of the nitrergic nervous system (mainly zaprinast), and to the drug treatment of erectile dysfunction (sildenafil). However, some 30–40% of patients suffering from impotence are still not adequately treated by sildenafil alone and therefore strategies to overcome this deficit are being pursued. In a recent study, it was shown that gene transfer of nitric oxide synthase into the corpus cavernosum of the aged rat enhanced the erectile response to nerve stimulation and to

zaprinast; thus, a combination of gene transfer therapy and phosphodiesterase 5 inhibitor might represent a novel and potentially more powerful treatment for erectile dysfunction (Champion et al., 1999). Experimental studies and clinical trials are also being carried out with combined drug therapy using phosphodiesterase 5 inhibitors with other drugs which promote penile erection (α-adrenoceptor antagonists, dopamine receptor agonists, prostanoids), although early results have indicated potential problems with priapism (Steers, 1999). Finally, the detection of different isoforms of phosphodiesterase 5 (Lin et al., 2000) raises the possibility of identifying isoform-specific inhibitors, allowing organ-specific enhancement of nitrergic function. Opportunities for effective therapeutic intervention may therefore become available not only in sexual dysfunction but in other diseases associated with impaired nitrergic function.

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