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REVIEW

Novel drug targets for the pharmacotherapy of benign prostatic hyperplasia (BPH)

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Benign prostatic hyperplasia (BPH) is the major cause of lower urinary tract symptoms in men aged 50 or older. Symptoms are not normally life threatening, but often drastically affect the quality of life. The number of men seeking treatment for BPH is expected to grow in the next few years as a result of the ageing male population. Estimates of annual pharmaceutical sales of BPH therapies range from $US 3 to 10 billion, yet this market is dominated by two drug classes. Current drugs are only effective in treating mild to moderate symptoms, yet despite this, no emerging contenders appear to be on the horizon. This is remarkable given the increasing number of patients with severe symptoms who are required to undergo invasive and unpleasant surgery. This review provides a brief background on prostate function and the pathophysiology of BPH, followed by a brief description of BPH epidemiology, the burden it places on society, and the current surgical and pharmaceutical therapies. The recent literature on emerging contenders to current therapies and novel drug targets is then reviewed, focusing on drug targets which are able to relax prostatic smooth muscle in a similar way to the α_1-adrenoceptor antagonists, as this appears to be the most effective mechanism of action. Other mechanisms which may be of benefit are also discussed. It is concluded that recent basic research has revealed a number of novel drug targets such as muscarinic receptor or P2X-purinoceptor antagonists, which have the potential to produce more effective and safer drug treatments.

Abbreviations

ATP, adenosine 5′-triphosphate; BPH, benign prostatic hyperplasia; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CRELD, cysteine-rich with epidermal growth factor (EGF)-like domain; CGRP, calcitonin gene-related peptide; CHO, Chinese hamster ovary; DHT, dihydrotestosterone; FAAH, fatty acid amidohydrolase; FDA, Food and Drug Administration; IP_3, inositol triphosphate; LHRH, luteinizing hormone releasing hormone; NHS, National Health System; PDE, phosphodiesterase; RAMP, receptor activity modifying protein; TUMT, transurethral microwave therapy; TUNA, transurethral needle ablation; TURP, transurethral resection of the prostate

Background: normal prostate growth, development and gland function

The human prostate gland undergoes two androgen-dependent major growth stages. The first occurs during foetal development, when the gland arises from outgrowths of the urethral epithelium. The second phase occurs at puberty when the gland reaches a weight of approximately 20 g (McConnel, 1995). The adult prostate gland is a sponge-like male accessory structure which indirectly facilitates fertilization by increasing sperm motility and providing nourishment by the expulsion of fluid into the urethra. The columnar epithelial cells which line the prostatic ducts continually produce secretions. These secretions are stored in the ducts until the smooth muscle surrounding the ducts contracts under autonomic control, to expel the prostatic fluid just prior to ejaculation. The major constituents of prostatic

fluid are polyamines, plasminogen, proteolytic enzymes, acid phosphatase, electrolytes, zinc, glucose and citric acid (Fair and Cordonnier, 1978; Zaichick *et al.*, 1996; Weisser *et al.*, 1997). Prostatic fluid, together with the fluid expelled from the seminal vesicle makes up the bulk of semen in which sperm is suspended to make up the ejaculate.

Background: benign prostatic hyperplasia (BPH)

With increasing age, alterations in hormone levels often result in the serious disorder known as benign prostatic hyperplasia (BPH) which is caused by an imbalance between cellular proliferation and apoptosis within the prostate. BPH initially manifests itself as microscopic nodules which progressively proliferate and enlarge to increase the mass of both the glandular and stromal prostatic tissue (Wilson, 1980). This abnormal third phase of prostate growth leads to an increase in smooth muscle tone and/or size of the gland. Because of the prostate's anatomical location surrounding the urethra, this growth is the major cause of bladder outlet and urethral obstruction in ageing men. Despite gross morphological and anatomical differences between prostate glands in humans and rodents, there appears to be many similarities with regard to their pharmacology and histochemistry making most laboratory animals of use as investigative models of prostate function.

Symptoms of BPH begin to appear in some men as early as age 40 and include a reduced urinary stream, nocturia, urinary retention, urgency, frequency and dribbling (Scarpa, 2001). These lower urinary tract symptoms and pathological BPH continue to worsen with age (Tsukamoto *et al.*, 2007). Diagnosis of BPH is generally by a combination of physical examination by a physician and patient self-assessment to determine a score of urinary symptom severity.

Because of the unique capsular anatomy of the human prostate, BPH is almost exclusively unique to humans. The only experimental animals to spontaneously develop BPH are dogs (no prostatic capsule) and chimpanzees (prostatic capsule), with only the latter developing the symptoms associated with BPH (Wilson, 1980). Nearly every man at some point in his life will be affected by BPH, with 90% of men over the age of 85 affected by the disease (Gallegos and Frazee, 2008). A recent study of 5990 Australian men reported that 16% of men over the age of 40 complained of moderate to severe lower urinary tract symptoms, with 19% reporting symptoms of nocturia which necessitated two or more voids per night. Above the age of 70 years, 38% reported prostate disease and 41% reported nocturia. Twenty-nine per cent of men over the age of 70 complained of moderate to severe symptoms (Holden *et al.*, 2005).

Background: burden on society of BPH

While BPH may be asymptomatic and undiagnosed in some men, obstruction of the urethra and deformation of the base of the bladder by the hyperplasic prostate will cause painful and debilitating lower urinary tract symptoms in most men as they age. Although rarely life threatening, the symptoms caused by BPH can have an extremely detrimental effect on the quality of life of both the men suffering from the disease as well as their partners. In the worst cases, sufferers may be forced to wake up to 10 times per night to urinate. Often, sufferers will also wake their partners as well. Such interrupted sleep patterns severely impact the ability of people to function normally during the day. Thus, BPH can reduce quality of life and drastically reduce the productivity of people affected by the disease. Because of the age-related nature of BPH progression, its prevalence will continue to increase in the short term as the population of western society ages.

Market research data for 2009 freely available on the Internet lists worldwide prostatic pharmacotherapy sales of $US 4.8 billion (Table 1). Nevertheless, pharmacological therapies are only effective for treating mild to moderate symptomatic cases of BPH. At present, patients experiencing severe symptoms can only be treated with the common but very invasive surgical procedures available such as transurethral resection of the prostate (TURP). More than 25 000 TURPs are performed in Australia each year at a cost of approximately $US 70 million (including public and private

Table 1

Top 4 marketed pharmacotherapy products for prostatic therapies 2009

Rank	Product	Generic name	Company	Annual worldwide sales $US (million)			Group share (%)		
				2007	2008	2009	2007	2008	2009
1	Flomax/Alna/Harnal	Tamsulosin HCl	Boehringer Ingelheim / Astellas Pharma	2171	2279	2692	48	48	50
2	Avodart/Avolve	Dutasteride	GlaxoSmithKline	571	739	830	14	17	17
3	Xatral/Uroxatral	Alfuzosin HCl	Sanofi-Aventis	457	486	413	11	11	9
4	Proscar	Finasteride	Merck & Co	411	324	291	10	8	6
Other				424	486	554	11	11	12
Total				4033	4313	4779	100	100	100

Source: EvaluatePharma® (http://www.evaluatepharma.com).

systems). The public system component alone represents 0.2% of total Medicare benefits paid (data obtained from National Hospital Morbidity Collection, Australian Institute of Health and Welfare). Similarly, in the United Kingdom, the economic burden of BPH to the National Health System (NHS) and Department of Social Security represents 0.4% of total NHS expenditure.

Current therapies: surgical treatments for BPH

Current treatment options for BPH include surgical and pharmacological therapies. In severe cases, potentially life-threatening complications may occur, such as acute urinary retention, recurrent infections and renal failure. In such cases, surgery is the only truly effective treatment. As well as TURP, other surgical procedures include transurethral microwave thermotherapy (TUMT) and transurethral needle ablation (TUNA) (Miano *et al.*, 2008). TURP is the most common surgical procedure and provides the greatest symptomatic relief, although it is a highly invasive procedure and side effects such as sexual dysfunction and incontinence are common. TUMT and TUNA are less invasive procedures and have fewer side effects, but are not as effective (Thorpe and Neal, 2003). For bothersome and enlarged prostates, open radical prostatectomy is also an option. However, nowadays this extremely invasive procedure is almost exclusively reserved for patients diagnosed with prostate cancer, which is beyond the scope of this review.

Current therapies: 5α-reductase inhibitor pharmacotherapy

Several recent reviews address the topic of future targets for the pharmacological treatment of BPH (Auffenberg *et al.*, 2009; Stamatiou, 2009; Hashim and Abrams, 2010). This review broadens the list of potential targets by summarizing in more detail the literature on prostate function in not only humans but also laboratory animals.

Current pharmacotherapy targets either the static (an increase in the physical size of the prostate) or dynamic (an increase in the tone of the smooth muscle) component of BPH. Drugs targeting the static component of BPH act by inhibiting the proliferative action of androgens. These drugs include finasteride (Proscar®, Merck, Whitehouse Station, NJ, USA) and dutasteride (Avodart®, GlaxoSmithKline, Brentford, Middlesex, UK) which make up 23% ($US 1.1 billion) of the prostate pharmacotherapy worldwide market (Table 1). This class of drugs inhibit the action of the 5α-reductase enzyme which catalyses the conversion of testosterone to the more potent androgen dihydrotestosterone (Figure 1). Urethral obstruction is therefore relieved as the physical size of the prostate is decreased (Carson and Rittmaster, 2003; Tarter and Vaughan, 2006). The main complaints relating to patient use of 5α-reductase inhibitors are that they are slow-acting, often taking up to 6 months before effective relief of symptoms, and are associated with sexual side effects including impotence, decreased libido and abnormal ejaculation.

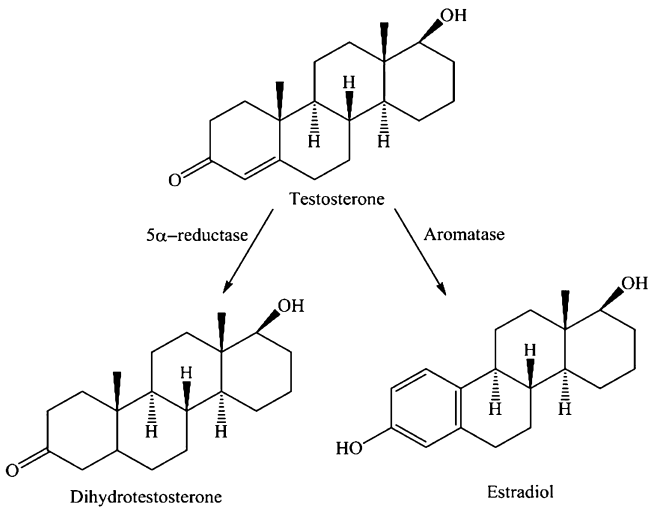

Figure 1

Chemical structure and metabolism pathway showing the steroid conversion of testosterone into dihydrotestosterone and estradiol, via 5α-reductase and aromatase respectively.

Current therapies: α_1-adrenoceptor antagonist pharmacotherapy

The most effective and fastest acting treatments for BPH are drugs targeting the dynamic component (Miano *et al.*, 2008). These include α_1-adrenoceptor antagonists such as tamsulosin (Flomax®, Yamanouchi, Tokyo, Japan; CSL, Parkville, VIC, Australia; Alna®, Boehringer Ingelheim am Rhein, Germany; Harnal®, Astellas, Tokyo, Japan) and alfuzosin (Uroxatral®, Sanofi-Aventis, Paris, France) which make up 65% ($US 3.1 billion) of the prostate therapy worldwide market (Table 1). These drugs decrease the stromal smooth muscle tone by blocking prostatic α_1-adrenoceptors (Figures 2 and 3), thus relieving urethral obstruction and the associated troublesome voiding symptoms (Lepor, 2007).

There is a direct correlation between urethral obstruction and the amount of prostatic smooth muscle (Shapiro *et al.*, 1992). In addition, 5α-reductase inhibitors are less effective than the α_1-adrenoceptor antagonists in the reduction of symptoms (Rigatti *et al.*, 2003; Hasan *et al.*, 2007; Lepor, 2007), indicating a greater importance for the dynamic component in the pathology of BPH. The relative importance of the dynamic component is further highlighted by observations that the severity of symptoms is not related to prostate size (Eckhardt *et al.*, 2001a,b,c). α_1-Adrenoceptor antagonists produce mainly vasodilator side effects, but selective blockade of the α_{1A}-adrenoceptor subtype is also associated with some abnormal ejaculatory effects (Rokosh and Simpson, 2002).

Emerging contenders to current therapies

α_1-Adrenoceptor antagonists and 5α-reductase inhibitors remain the major targets of new drugs for the treatment of

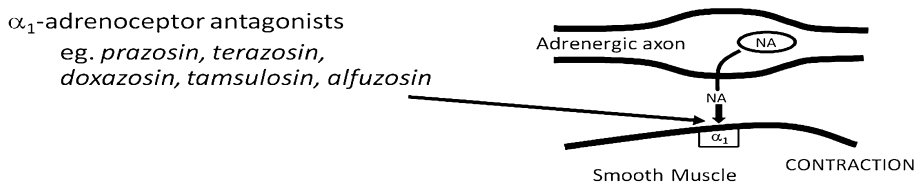

Figure 2

Schematic diagram showing the postjunctional site of action of the α_1-adrenoceptor antagonists as smooth muscle relaxants in the prostate gland.

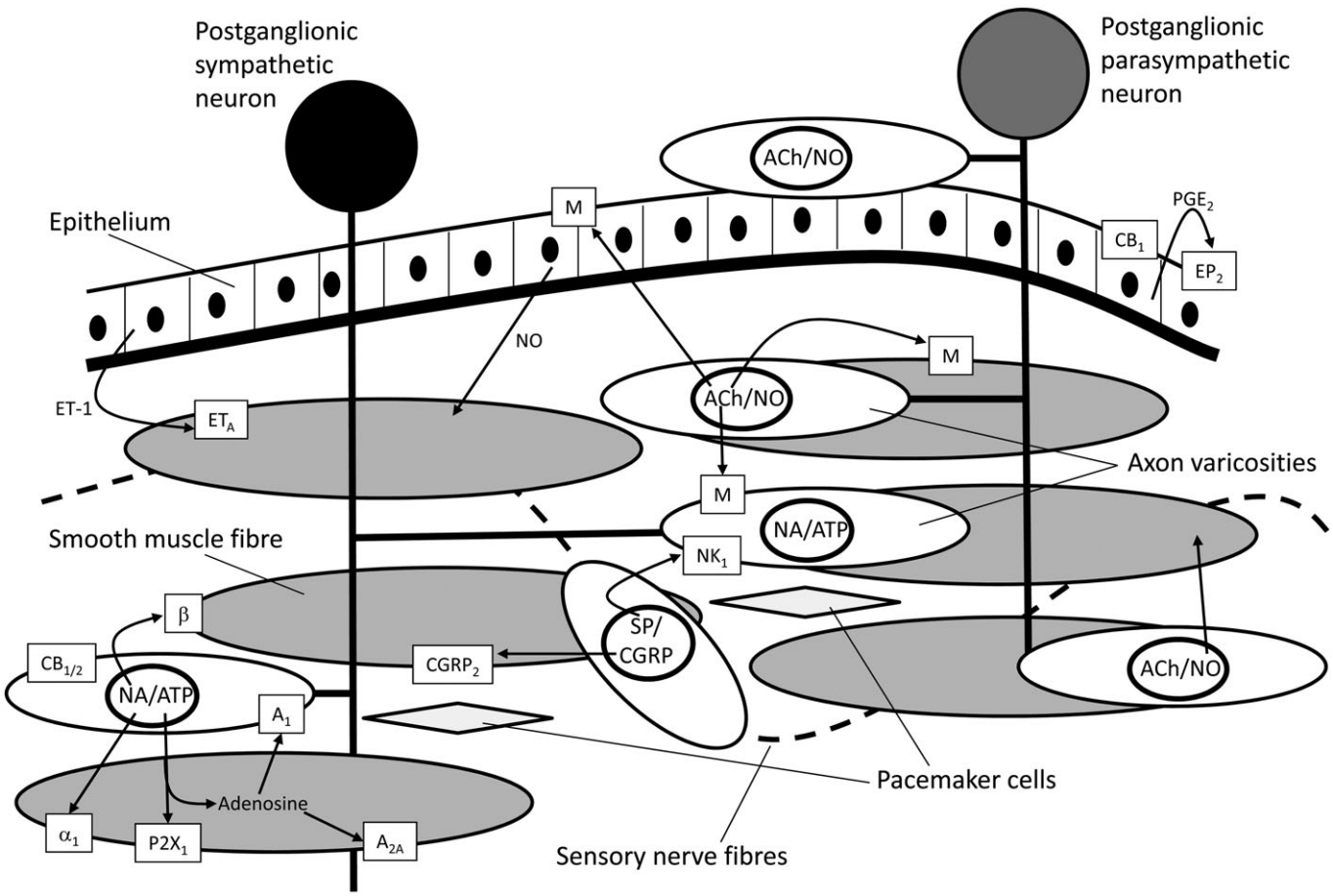

Figure 3

Schematic diagram showing the likely locations on various cell types of membrane bound receptors and extracellular messengers which play a role in the control of contractility of prostatic smooth muscle. The diagrammatic representation is derived from experimental data obtained in a number of species. ACh, acetylcholine; ATP, adenosine 5′-triphosphate; CGRP, calcitonin gene-related peptide; ET, endothelin; NA, noradrenaline; NO, nitric oxide; SP, substance P.

BPH. Most pharmaceutical research centres on making new compounds which will more selectively act at these targets and reduce adverse side effects, thereby improving patient adherence. Despite the effectiveness of current BPH treatments, there still remains the opportunity for new therapies that can stop disease progression and the subsequent need for surgery. The therapeutic potential for a combination therapy of 5α-reductase inhibitors and α_1-adrenoceptor antagonists is also very apparent. Combined use leads to a decreased progression of BPH, as well as mild symptomatic relief (Schulman, 2003). Indeed, in June 2010, a single-capsule formulation of dutasteride and tamsulsion (Jalyn®, GSK) was approved by the US Food and Drug Administration for use in symptomatic BPH in men with an enlarged prostate.

Gonadotrophin modulators, in particular, luteinizing hormone releasing hormone (LHRH) receptor antagonists such as cetrorelix have been extensively trialled for the treatment of BPH. The rationale for their use is based on inhibiting LHRH receptors in the pituitary, thereby blocking the signal for testosterone production in the testes and conse-

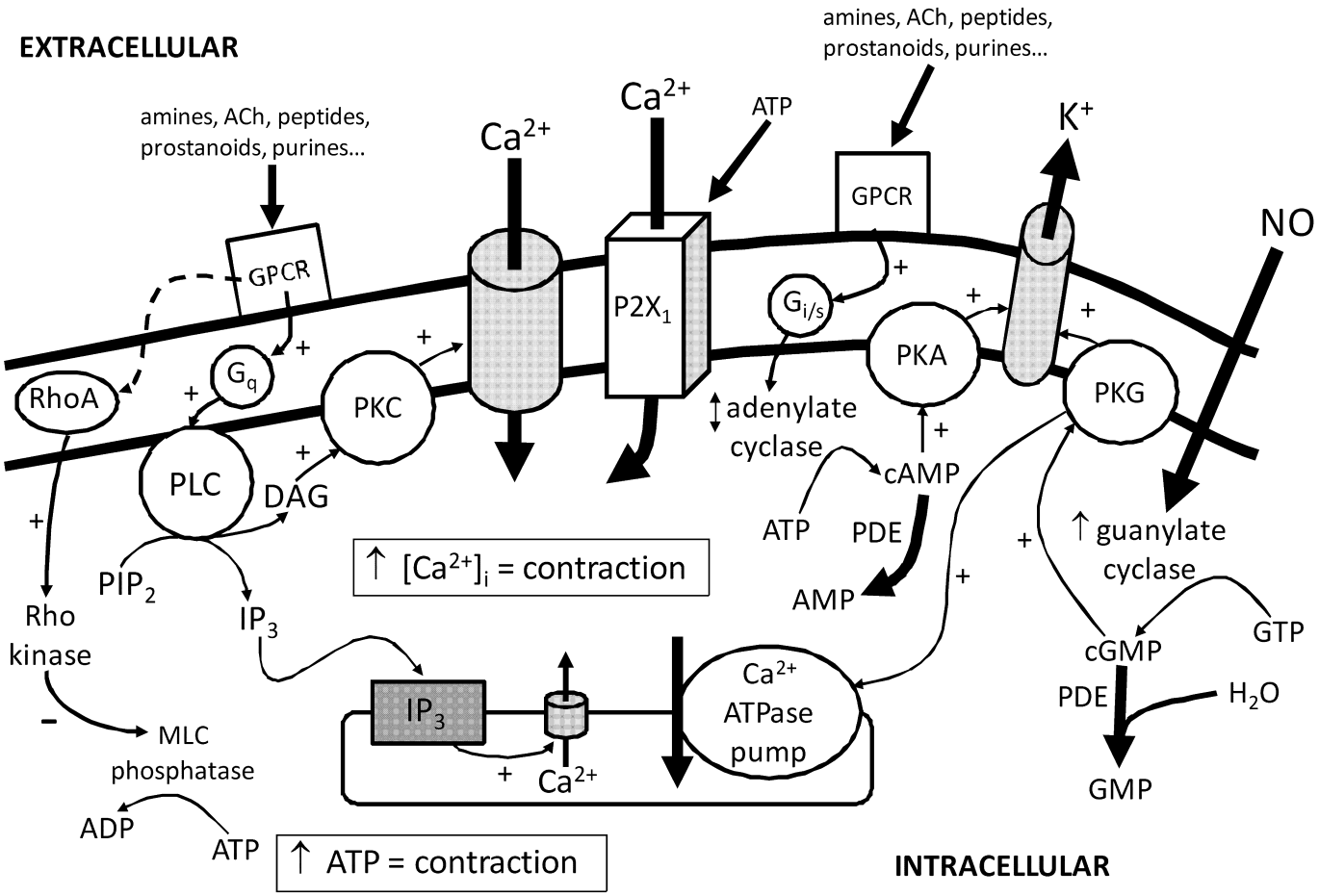

Figure 4

Schematic diagram of the likely intracellular signalling pathways at play in controlling contractility of prostatic smooth muscle cells. ACh, acetylcholine; Adr, adrenaline; AMP, adenosine monophosphate; ATP, adenosine 5′-triphosphate; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; DAG, diacylglycerol; GMP, guanosine monophosphate; GPCR, G-protein coupled receptor; IP_3, inositol triphosphate; MLC, myosin light chain; NO, nitric oxide; PDE, phosphodiesterase; PIP_2, phosphatidylinositol bisphosphate; PK, protein kinase; PL, phospholipase.

quently inhibiting prostate growth. Clinical studies have shown that cetrorelix decreases prostate volume and improves lower urinary tract symptoms in patients suffering from BPH (Gonzalez-Barcena *et al.*, 1994; Comaru-Schally *et al.*, 1998; Debruyne *et al.*, 2008, 2010). However, despite mild long-term symptomatic improvement, serum testosterone levels decrease only during dosing, and return to baseline levels in a matter of weeks (Comaru-Schally *et al.*, 1998). This suggests that LHRH antagonists may act directly on the prostate to inhibit growth. On the contrary, LHRH agonists have been shown to have anti-proliferative effects in both androgen-dependent and independent prostate cancer cell lines (Limonta *et al.*, 1992; 1999; Dondi *et al.*, 1994). However, in the non-tumourgenic epithelial line BPH-1 (derived from prostate tissue of a 68-year-old BPH patient), the LHRH antagonist, cetrorelix, inhibits cell proliferation and reduces expression of mitogenic growth factors (Siejka *et al.*, 2010). In a rat model of BPH, cetrorelix has also been shown to reduce prostate size presumably by inhibiting pro-inflammatory cytokines and growth factors (Rick *et al.*, 2011).

Because of either their direct or indirect effects on prostate growth, LHRH receptors represent an emerging drug target for the treatment of BPH. However, their efficacy appears only mild, and the amount of symptomatic relief that they can provide remains to be seen. Furthermore, they are associated with generally unacceptable anti-androgenic side effects such as impotence, which has led to a number of pharmaceutical companies abandoning this approach to treat BPH.

Novel vitamin D3 analogues such as BXL-628 have also shown efficacy in arresting prostate growth in patients with BPH in recent clinical trials (Colli *et al.*, 2006). In this study, there was a small percentage decrease of approximately 7% in prostate size compared with placebo over 3 months of treatment. However, this decrease in prostate size did not translate to an improvement in urinary symptom score. *In vitro* studies have also shown BXL-628 to inhibit RhoA/Rho kinase signalling, which is an important intracellular pathway in smooth muscle cell contractility (Figure 4) (Morelli *et al.*, 2007). However, the observed delay in carbachol-induced smooth muscle contraction was only minimal, and the lack of effect

on patient urinary symptom score indicates that BXL-628 may not have the efficacy to compete with currently available treatments. On the other hand, carbachol is a non-selective muscarinic receptor agonist which suggests that it may only partially produce its effect by interaction with the RhoA/Rho kinase pathway. The merits of the RhoA/Rho kinase signalling pathway as a therapeutic target for BPH will be further discussed later in this review.

Novel targets: β-adrenoceptor agonists

There are surprisingly few studies investigating the effects of β-adrenoceptors on human prostate contractility, given their seemingly large efficacy (Table 3). Early studies, attempting to characterize β-adrenoceptor activity in human prostate tissues met with little success (Caine *et al.*, 1975). As the pharmacological tools developed, further attempts to classify prostatic β-adrenoceptors arose. β-Adrenoceptor-mediated elevations of adenylate cyclase were seen in human prostate 'stromal' and 'epithelial' tissues (Purvis *et al.*, 1986). Later, it was shown that the non-selective antagonist, propranolol enhanced responses to both transmural stimulation and exogenously applied noradrenaline, and that this effect was more pronounced in tissue from non-hyperplastic tissues, which also showed a reduction in dihydroalprenolol binding sites (Tsujii *et al.*, 1992). In the mid-1990s, β_3-adrenoceptor mRNA was identified in human prostatic tissue (Berkowitz *et al.*, 1995), while more radioligand binding studies showed a dominance of the β_2- over the β_1-adrenoceptor subtype (Goepel *et al.*, 1997). Subsequently, a β_3-adrenoceptor antibody appeared to localize the β_3-adrenoceptor to a subpopulation of prostatic stromal cells (Chamberlain *et al.*, 1999). Functionally, the β_3-adrenoceptor appears to be a better target for pharmaceutical agents as its distribution appears more restricted than that of the β_1- and β_2-adrenoceptors. In prostatic stromal cells derived from human prostatic tissue, β_3- and possibly β_1-adrenoceptor activation reduces α_1-adrenoceptor-mediated contractions (Haynes and Hill, 1997), although animal studies show a predominant role for β_2- and/or β_1-adrenoceptors (Haynes and Hill, 1997; Kalodimos and Ventura, 2001). The most significant indication that β-adrenoceptors may prove a suitable therapeutic target is data suggesting that β blocker therapy increases the risk of developing BPH (Meigs *et al.*, 2001).

Novel targets: muscarinic receptor antagonists

Muscarinic receptor antagonists are not really novel treatments for BPH, but their use is limited by theoretical concerns that such drugs may lead to an increase in post-void residual, voiding difficulties and acute urinary retention due to detrusor inhibition. Recent reviews of clinical trials using muscarinic receptor antagonists (e.g. tolterodine, solifenacin, propiverine, oxybutynin or darifenacin) for the treatment of lower urinary tract symptoms, usually in combination with an adrenoceptor antagonist (e.g. doxazosin, tamsulosin or terazosin), show varying improvement in storage symptoms without worsening of voiding symptoms or significant development of acute urinary retention (Athanasopoulos, 2010; Chapple, 2010). This suggests that these drugs do indeed act by inhibition of detrusor instability in the bladder. Whether or not these antagonists also act in the prostate to relieve bladder outlet obstruction remains unclear.

Early studies found that compared with adrenoceptor-mediated contraction, muscarinic receptor agonists elicited only modest contractile responses from the human prostatic capsule. These responses were absent from the prostatic adenoma and did not enhance or inhibit adrenoceptor-mediated contraction (Caine *et al.*, 1975; Gup *et al.*, 1989; Kester *et al.*, 2003). A more recent study observed that carbachol enhanced the contractile response to phenylephrine (Roosen *et al.*, 2009). Furthermore, phenylephrine significantly reduced the pEC_{50} for carbachol-mediated contractions. The authors suggested that a significant adreno-muscarinic receptor synergy in the prostate exists which may contribute to bladder outflow resistance.

A recent study in the mouse prostate showed that after inhibition of the adrenergic contractile response, a large cholinergic component remained (White *et al.*, 2010). Moreover, a single randomized clinical trial investigating the safety and tolerability of tolterodine for the treatment of overactive bladder in patients with BPH indicated that there was a significant reduction in the bladder outlet obstruction index; however, the study provided no discussion of this result (Abrams *et al.*, 2006).

Finally, it should be noted that muscarinic receptor agonists can induce proliferation in a number of prostate cancer cell lines (Witte *et al.*, 2008; Avellar *et al.*, 2009). Therefore, as well as inhibiting detrusor overactivity, muscarinic receptor antagonists may also relieve voiding symptoms of BPH by decreasing the size of the prostate and decreasing prostatic smooth muscle tone.

Novel targets: $P2X_1$-purinoceptor antagonists

Adenosine 5′-triphosphate (ATP) has been shown to be an excitatory co-transmitter with noradrenaline from the sympathetic nerves innervating the prostate of both the rat (Ventura *et al.*, 2003) and guinea-pig (Buljubasich and Ventura, 2004). Despite the presence of several P2X-purinoceptor subtypes in the prostatic smooth muscle of mice (Gray and Ventura, 2005), rats (Lee *et al.*, 2000) and guinea-pigs (Buljubasich and Ventura, 2004), both studies also used pharmacological methods to demonstrate that the P2X-purinoceptor responsible for prostatic contraction was of the $P2X_1$-purinoceptor subtype. In both studies, blockade of $P2X_1$-purinoceptors with either suramin or α, β-methylene ATP was able to inhibit electrically evoked nerve-mediated contractions in addition to the inhibition produced by prazosin. The relevance of these findings involving ATP to human prostate contractility is questionable, but ecto 5′-nucleotidase, an enzyme responsible for the catabolism of ATP, has been shown to be present in the human prostate (Konrad *et al.*, 1998). Furthermore, $P2X_1$-purinoceptors are also expressed in human prostate (Longhurst *et al.*, 1996).

Functional studies in human prostate have shown that the contractile response to electrical field stimulation is almost completely suppressed by α-adrenoceptor antagonists (Hedlund *et al.*, 1985; Guh *et al.*, 1995; Chueh *et al.*, 1996). These studies were limited in that they used only high frequency stimulation (>2 Hz). Studies using prostates from laboratory animals have shown that purinergic neurotransmission is frequency dependent and particularly important at lower concentrations (Ventura *et al.*, 2003; Buljubasich and Ventura, 2004). Furthermore, the human studies had the limitation of tissue being solely obtained at surgery. Many prostate surgery techniques cause trauma to the nerves which causes compensatory phenotype changes. Nerves containing ATP are thought to be particularly susceptible to such plasticity of purinoceptor expression (Burnstock and Knight, 2004) which may dampen their involvement in human tissue studies.

The importance of ATP as a neurotransmitter in male genitourinary function can not be underestimated as $P2X_1$-purinoceptor knockout mice are known to be infertile (Mulryan *et al.*, 2000). ATP has recently been found to be involved in neurotransmission in the human vas deferens (Banks *et al.*, 2006). If ATP is similarly involved in neurotransmission in the human prostate, the development of selective inhibitors at the $P2X_1$-purinoceptor may provide an additional target. When used in combination with a α_{1A}-adrenoceptor antagonist, a $P2X_1$-purinoceptor antagonist could provide greater prostatic smooth muscle relaxation and therefore, greater relief from symptoms. There is also convincing evidence for a role of ATP at genitourinary P2X-purinoceptors in the human bladder, particularly in those displaying pathological contractile overactivity (Fry *et al.*, 2010). Therefore, it is likely that drugs affecting this mechanism would also act at the level of the bladder.

Novel targets: adenosine receptor agonists

Evidence exists that adenosine may modulate prostate smooth muscle contractility at two sites (Figure 3). In isolated rat prostates, activation of prejunctional A_1 adenosine receptors attenuates electrical field stimulation-induced contractile responses by inhibiting noradrenaline release (Preston *et al.*, 2000). In human prostatic stromal cells, activation of postjunctional A_{2A} adenosine receptors inhibits α_1 adrenoceptor-mediated responses via stimulation of adenylate cyclase and subsequent accumulation of cyclic adenosine monophosphate (cAMP) (Preston *et al.*, 2004). The physiological relevance of these observations is questionable, as gene disruption of the A_{2A} adenosine receptor in mice also produces a small reduction in prostate contractility (Gray *et al.*, 2008b). The major effects that adenosine has on the cardiovascular system also places doubt on whether adenosine receptors would be of any clinical value as a target for the treatment of BPH.

Novel targets: cannabinoids

Stimulation of CB_1 cannabinoid receptors have been shown to inhibit contractions of the rat prostate gland (Tokanovic *et al.*, 2007) (Tables 2 and 3). This action appeared to be indirect, and the authors used immunohistochemical techniques to localize the CB_1 cannabinoid receptors to the prostatic epithelium. CB_1 cannabinoid receptor expression has also been shown in the epithelial layer of the human prostate (Ruiz-Llorente *et al.*, 2003). Furthermore, an anandamide uptake transporter and the fatty acid amidohydrolase enzyme, which degrades endocannabinoids, are also expressed in the human prostate (Ruiz-Llorente *et al.*, 2004). More recently, CB_1 and CB_2 cannabinoid receptors have also been localized on sensory nerves innervating the human prostatic stroma and have been implicated in mediating inhibition of contraction (Gratzke *et al.*, 2010). This indicates that cannabinoid receptors may also be a possible therapeutic target for the treatment of BPH. However, their inhibitory effects on prostate contractility appear to be only modest (Table 3) (Tokanovic *et al.*, 2007; Gratzke *et al.*, 2010), and the effects of cannabinoid receptor agonist treatment on the central nervous system would also need to be considered.

Novel targets: prostaglandin E_2

Prostaglandin E_2 has been shown to inhibit contractions of the rat prostate gland through action at a prostanoid receptor of the EP_2 subtype (Tokanovic *et al.*, 2010) (Tables 2 and 3). Interestingly, prostaglandins were first isolated from semen and are so named because they were believed to be derived from the prostate gland (Bergstrom *et al.*, 1968). The effects of prostaglandins on the contractility of the prostate gland are not well characterized. Early studies indicated that some of the prostaglandins caused an excitatory contractile response (Kitada and Kumazawa, 1987; Najbar-Kaszkiel *et al.*, 1997; Sudoh *et al.*, 1997). Nevertheless, there is now mounting evidence that inflammatory roles may play a role in the development of BPH (Kramer *et al.*, 2007; Sciarra *et al.*, 2007; 2008). Despite this, prostanoids have very widespread effects throughout the body (Narumiya *et al.*, 1999) which may limit their use in the treatment of BPH.

Novel targets: histamine

Histamine has been shown to contract dog prostate (Normandin and Lodge, 1996) as well as have excitatory effects on Ca^{2+} mobilization in prostate cancer cells (Wasilenko *et al.*, 1997). A later study in guinea pig prostate showed that histamine was not able to cause contraction on its own but potentiated contractions in response to electrical nerve stimulation and the exogenous application of ATP, noradrenaline or acetylcholine (Kerr, 2006). This potentiation appeared to be mediated by H_1 histamine receptors. The clinical significance of these findings for the treatment of BPH is unclear as the H_1 histamine receptor antagonist mepyramine on its own appeared to have no effect on contractions mediated by electrical field stimulation (Tables 2 and 3).

Novel targets: peptides

A number of peptides have been shown to have varying effects on the contractility of the prostate gland in a number

Table 2

Receptor mechanisms mediating contractile and relaxant effects of prostatic stroma

Receptor	Agonists	Antagonists	Species	References
α_1-adrenoceptor	↑	↓	Mouse	Gray and Ventura (2005)
				Gray and Ventura (2006)
			Rat	Najbar-Kaszkiel *et al.* (1997)
			Guinea-pig	Najbar-Kaszkiel *et al.* (1997)
				Pennefather *et al.* (1999)
			Rabbit	Couldwell *et al.* (1993)
				Testa *et al.* (1993)
				Yazawa and Honda (1993)
				Hiraoka *et al.* (1995)
				Delaflotte *et al.* (1996)
				Najbar-Kaszkiel *et al.* (1997)
			Dog	Ohmura *et al.* (1993)
				Normandin and Lodge (1996)
			Human	Testa *et al.* (1993); Testa *et al.* (1996)
				Chapple *et al.* (1994)
				Muramatsu *et al.* (1994)
				Teng *et al.* (1994)
				Eckert *et al.* (1995)
				Guh *et al.* (1995)
				Marshall *et al.* (1995)
				Tseng-Crank *et al.* (1995)*
β_2-adrenoceptor	↓	–	Rat	Kalodimos and Ventura (2001)
			Guinea-pig	Haynes and Hill (1997)
			Dog	Normandin and Lodge (1996)
			Human	Tsujii *et al.* (1992)
Muscarinic receptor	↑	↓	Mouse	White *et al.* (2010)
			Rat	Najbar-Kaszkiel *et al.* (1997)
				Lau and Pennefather (1998)
				Lau *et al.* (1998)
			Guinea-pig	Najbar-Kaszkiel *et al.* (1997)
				Lau *et al.* (1998)
				Lau *et al.* (2000)
			Rabbit	Najbar-Kaszkiel *et al.* (1997)
			Dog	Normandin and Lodge (1996)
				Fernandez *et al.* (1998)
$P2X_1$-purinoceptor	↑	↓	Rat	Ventura *et al.* (2003)
			Guinea-pig	Buljubasich and Ventura (2004)
A_1-adenosine receptor	↓	–	Rat	Preston *et al.* (2000)
NK-tachykinin receptor	↑	–	Guinea-pig	Buljubasich *et al.* (1999)
			Human	Palea *et al.* (1996)
Calcitonin gene-related peptide receptor	↓	–	Rat	Watts and Cohen (1991)
				Ventura *et al.* (2000b)
ET_A-endothelin receptor	↑	–	Rat	Salamoussa *et al.* (2000)
			Guinea-pig	Lau *et al.* (1999)
			Dog	Normandin and Lodge (1996)
				Langenstroer *et al.* (1997)
				Imajo *et al.* (1997)
			Human	Langenstroer *et al.* (1993)
				Webb *et al.* (1995)*
				Moriyama *et al.* (1996)
				Imajo *et al.* (1997)
				Raschack *et al.* (1998)
CB-cannabinoid receptors	↓	–	Rat	Tokanovic *et al.* (2007)
			Human	Gratzke *et al.* (2010)
EP_2-prostanoid receptor	↓	–	Rat	Tokanovic *et al.* (2010)
Histamine receptors	↓	–	Guinea-pig	Kerr (2006)
			Dog	Normandin and Lodge (1996)

Upward arrows indicate an overall excitatory effect on contractility indicated by either an increase in basal tone or an enhancement of electrically evoked contractions.
Downward arrows indicate an overall inhibitory effect on contractility indicated by a relaxation of precontracted tissue or inhibition of electrically evoked contraction.
Dashes indicate no effect on contractility elicited by electrical stimulation or exogenous addition of α-adrenoceptor agonists.
All cited studies used direct force measurements of isolated prostate tissue preparations unless indicated by an asterisk*.

Table 3

Relative efficacies of various drug classes in relaxing isolated prostatic smooth muscle preparations precontracted by electrical stimulation or exogenous application of adrenoceptor agonists (where indicated by an asterisk*)

Drug class	Contraction remaining (%)	Species	References
Adenosine receptor agonist	~20	Rat	Preston *et al.* (2000)
CGRP	~60*	Rat	Watts and Cohen (1991)
	~45	Rat	Ventura *et al.* (2000b)
α-adrenoceptor antagonist	~65	Mouse	Gray and Ventura (2005)
	~50	Mouse	White *et al.* (2010)
	~40	Rat	Najbar-Kaszkiel *et al.* (1997)
	~45	Rat	Ventura *et al.* (2003)
	~50	Guinea-pig	Buljubasich and Ventura (2004)
	~40	Guinea-pig	Najbar-Kaszkiel *et al.* (1997)
	~50	Rabbit	Najbar-Kaszkiel *et al.* (1997)
	~90	Pig	Najbar-Kaszkiel *et al.* (1997)
β-adrenoceptor agonist	~60	Rat	Kalodimos and Ventura (2001)
	~60*	Guinea-pig	Haynes and Hill (1997)
PGE_2	~65	Rat	Tokanovic *et al.* (2007)
	~65	Rat	Tokanovic *et al.* (2010)
Nitric oxide donors	100*	Rat	Najbar-Kaszkiel *et al.* (1997)
	~70*	Guinea-pig	Najbar-Kaszkiel *et al.* (1997)
	~75*	Rabbit	Najbar-Kaszkiel *et al.* (1997)
	~70*	Pig	Najbar-Kaszkiel *et al.* (1997)
	~45*	Human	Hedlund *et al.* (1997)
CB-cannabinoid receptor agonist	~75	Rat	Tokanovic *et al.* (2007)
	<73*	Human	Gratzke *et al.* (2010)
P2X-purinoceptor blocker	100	Mouse	Gray and Ventura (2005)
	100	Mouse	White *et al.* (2010)
	~70	Rat	Ventura *et al.* (2003)
	~70	Guinea-pig	Buljubasich and Ventura (2004)
Muscarinic receptor antagonist	~80	Mouse	Gray and Ventura (2005)
	~75	Mouse	White *et al.* (2010)
	100	Rat	Najbar-Kaszkiel *et al.* (1997)
	~85	Guinea-pig	Najbar-Kaszkiel *et al.* (1997)
	~80	Rabbit	Najbar-Kaszkiel *et al.* (1997)
	100	Pig	Najbar-Kaszkiel *et al.* (1997)
ET-endothelin receptor antagonist	100	Rat	Salamoussa *et al.* (2000)
	100	Guinea-pig	Lau *et al.* (1999)
Histamine receptor antagonist	100	Guinea-pig	Kerr (2006)
NK-tachykinin receptor antagonist	100	Guinea-pig	Buljubasich *et al.* (1999)

Estimates of efficacy are derived from published experimental data (often estimated from figures) in prostates taken from various species using various drugs.
CGRP, calcitonin gene-related peptide; PGE_2, prostaglandin E_2.

of species (Table 2). Tachykinins have been demonstrated to be able to produce tonic contractions of isolated preparations of human prostate via the stimulation of NK_2 tachykinin receptors (Palea *et al.*, 1996). Similar effects on smooth muscle have not been reported in rodent models, but tachykinins potentiate electrical field stimulation induced contractile responses of prostates taken from guinea pigs through stimulation of NK_1 tachykinin receptors (Buljubasich *et al.*, 1999; Ventura *et al.*, 2000a). In stark contrast, tachykinins have no effect on prostates taken from rats despite the presence of nerves immunoreactive for substance P and neurokinin A (Buljubasich *et al.*, 1999).

Conversely, the sensory neuropeptide calcitonin gene-related peptide (CGRP) has been shown to inhibit contractions of the rat prostate elicited by exogenous administration of phenylephrine (Watts and Cohen, 1991) or electrical field stimulation (Ventura *et al.*, 2000a,b) (Tables 2 and 3). In further contrast to the tachykinins, CGRP has no effect on contractility of prostates obtained from guinea-pigs despite the presence of CGRP immunoreactive nerves (Ventura *et al.*, 2000b). The effects of CGRP are yet to be tested in human prostate despite numerous reports of CGRP immunoreactivity (Chapple *et al.*, 1991; Jen and Dixon, 1995; Tainio, 1995; Hedlund *et al.*, 1997). If similar smooth muscle relaxant effects were seen to CGRP, then this peptide may be potentially of some use in the treatment of BPH.

Similar to the sensory neuropeptides, endothelins also show species biodiversity in prostates taken from laboratory animals. In guinea-pigs, endothelins potentiate nerve-mediated contractions without affecting basal smooth muscle tone (Lau *et al.*, 1999), whereas in rat prostates endothelins raise smooth muscle tone without affecting nerve mediated contractions (Salamoussa *et al.*, 2000). In contrast to the neuropeptides, endothelins have been widely studied in human prostate. Endothelin-1 immunoreactivity has been demonstrated in the glandular epithelium (Langenstroer *et al.*, 1993), and cultured cells derived from human prostate epithelium have been shown to secrete endothelin-1 (Walden *et al.*, 1998). Endothelin-1 has also been shown to have contractile effects in human prostate (Langenstroer *et al.*, 1993; Moriyama *et al.*, 1996; Imajo *et al.*, 1997). As in all animal species studied (Table 2), contractile effects of endothelins in the normal human prostate are mediated via ET_A endothelin receptors which are found throughout the human prostatic stroma (Kobayashi *et al.*, 1994). However, in BPH, the subtype of endothelin receptor which mediates contraction may change to ET_B (Webb *et al.*, 1995), as the number of endothelin receptors increases dramatically (Kondo *et al.*, 1994; 1995; Moriyama *et al.*, 1996). Endothelins also promote growth of human cultured smooth muscle prostatic cells through both ET_A and ET_B subtypes of endothelin receptor (Saita *et al.*, 1998). This suggests that endothelins may play an excitatory role in proliferation as well as contractility in the human prostate. Such a combination of actions would theoretically be advantageous in a BPH drug target; however, antagonists of endothelin receptors have been shown to have little or no effect on contractility on their own (Tables 2 and 3).

Novel targets: α-adrenoceptor interacting proteins

Recent work investigating the changed pharmacology of the α_{1A}-adrenoceptor in the prostate gland has led to the possibility that an interacting protein is associated with this receptor in this tissue. With the use of genetically modified knockout mice, it has been shown in our laboratory that the α_{1L}-adrenoceptor pharmacological phenotype observed in the prostate arises from the α_{1A}-adrenoceptor gene (Gray *et al.*, 2008a) and this has since been confirmed by other researchers (Muramatsu *et al.*, 2008). Initially, it was thought that a polymorphism or splice variant of the α_{1A}-adrenoceptor gene may lead to the pharmacological expression of the α_{1L}-adrenoceptor functional phenotype, but this was shown not to be the case by a number of different groups (Shibata *et al.*, 1996; Suzuki *et al.*, 2000; Ramsay *et al.*, 2004). Using whole segments as well as homogenates of prostate tissue in radioligand binding experiments to support their theory, it has now been postulated that an interacting protein may be associated with the α_{1A}-adrenoceptor in tissues where α_{1L}-adrenoceptor pharmacology is exhibited (Muramatsu *et al.*, 2005; Nishimune *et al.*, 2010a). This may explain why α_{1L}-adrenoceptor pharmacology is only seen in functional studies using intact tissue or whole cells and not in radioligand binding experiments where prostate tissue homogenates have been used (Muramatsu *et al.*, 2005; Nishimune *et al.*, 2010a).

A yeast two-hybrid approach to screen the human prostate cDNA library using the full open reading frame of the human α_{1A}-adrenoceptor gene as bait was recently used to identify cysteine-rich with epidermal growth factor-like domain 1α (CRELD1α) as a protein that interacts with the α_{1A}-adrenoceptor, and may account for the emergence of α_{1L}-adrenoceptor pharmacology in certain cell types (Nishimune *et al.*, 2010b). Co-expression of CRELD1α with the α_{1A}-adrenoceptor generates a larger proportion of receptors displaying α_{1L} pharmacology in Chinese hamster ovary cells (Nishimune *et al.*, 2010b). However, the apparent abundance of adrenoceptors also changed markedly, so it is not clear whether CRELD1α is able to change the properties of this adrenoceptor by modifying its conformation (as seen with receptor activity modifying proteins), simply reducing its expression or changing its localization within the cell.

The possibility of a protein which interacts with the α_{1A}-adrenoceptor to change its pharmacology is plausible and would be a logical explanation for the diverse α_{1A}-adrenoceptor pharmacology seen in the prostate gland. CRELD1α may or may not be such a protein; however, identification of a α_{1A}-adrenoceptor interacting protein or a α_{1A}-adrenoceptor heteromer complex with another receptor which changed its pharmacology would be a significant advance in developing a more prostate-specific α_{1A}-adrenoceptor antagonist.

Novel targets: phosphodiesterase (PDE) inhibitors

The major intracellular signalling pathways involved in prostatic smooth muscle cell contraction are shown in Figure 4. Of these, phosphodiesterase (PDE) would appear to be the most appropriate drug target for the treatment of BPH. PDEs play an important role in terminating signalling through the breakdown of cyclic nucleotide monophosphates [cAMP and cyclic guanosine monophosphate (cGMP); Figure 4]. Early studies showed that both direct or indirect activators of the cyclic nucleotide stimulating pathway relaxed prostate tissue (Drescher *et al.*, 1994; Haynes and Cook, 2006; Kedia *et al.*, 2006; Oger *et al.*, 2009), an effect possibly mediated through the activation of potassium channels (Kurokawa *et al.*, 1998; Cook *et al.*, 2002; Haynes and Cook, 2006). Cyclic nucleotides and PDE inhibitors also modulate human prostatic cell

proliferation (Guh *et al.*, 1998; Adolfsson *et al.*, 2002; Cook and Haynes, 2004; Fibbi *et al.*, 2010) and fibroblast-to-myofibroblast transdifferentiation (Zenzmaier *et al.*, 2010).

Prostatic tissue is reported to have at least 14 isozymes (Stacey *et al.*, 1998; Uckert *et al.*, 2001), but the major isozymes appear to be the PDE4A, 4B, 5A and 11A4 (Fawcett *et al.*, 2000; Yuasa *et al.*, 2000) which have varied, and occasionally opposing, distributions throughout the glandular and stromal compartments (Uckert *et al.*, 2006; Fibbi *et al.*, 2010; Waldkirch *et al.*, 2010).

Largely, as an indirect result of current treatments for erectile dysfunction, there is clinical data suggesting that PDE inhibitors may be of benefit in treating lower urinary tract symptoms. For example, the administration of two PDE5 inhibitors, adalafil or udenafil, increases prostatic cAMP and cGMP (Zhao *et al.*, 2011). More importantly, the PDE5 inhibitors, tadalafil, sildenafil and vardenafil improve prostate symptom scores (McVary *et al.*, 2007a,b; 2008; Roehrborn *et al.*, 2008; Stief *et al.*, 2008; Dmochowski *et al.*, 2010; Tuncel *et al.*, 2010). Whether PDE5 inhibitor therapies provide more relief from lower urinary tract symptoms alone, than in combination with other therapies, is still equivocal (Kaplan and Gonzalez, 2007; Kaplan *et al.*, 2007; Kaplan and Hatzichristou, 2007; Tuncel *et al.*, 2010).

Novel targets: RhoA/Rho kinase

The RhoA/Rho kinase pathway has received much attention in recent years due to its role in regulating smooth muscle contractility. RhoA is a G-protein coupled to excitatory receptors (e.g. α_1-adrenoceptors), which activates Rho kinase, which in turn maintains actin filaments in a contracted state through inhibition of myosin light chain phosphatase (Figure 4). RhoA and Rho kinase are expressed in smooth muscle cells of the human prostate, and inhibitors of Rho kinase decrease contractile responses to electrical field stimulation, noradrenaline, phenylephrine and endothelin in human prostate tissue (Takahashi *et al.*, 2007) or cultured prostatic stromal cells (Rees *et al.*, 2003). Interestingly, inhibitors of Rho kinase have also been found to decrease cell proliferation (Rees *et al.*, 2003). Although studies on the efficacy of Rho kinase inhibitors are yet to be carried out in a clinical setting, the RhoA/Rho kinase pathway shows potential as a therapeutic target for controlling both the increased growth and contractility associated with BPH. Indeed, this pathway has been shown to be the mechanism of action of a number of novel compounds which show potential in BPH treatment, such as the vitamin D receptor agonist BXL-628 (Morelli *et al.*, 2007), KMUP-1 (Liu *et al.*, 2007) and isoflavones (Seok *et al.*, 2008).

Novel targets: nitric oxide

The human prostate stroma receives dense nitrergic innervation which has been found to be reduced in BPH patients (Bloch *et al.*, 1997). Studies have shown that nitric oxide donors inhibit noradrenaline-mediated contractions and induce smooth muscle relaxation in human prostate tissue (Hedlund *et al.*, 1997) (Table 3). Similarly, inhibitors of nitric oxide synthesis have been found to decrease electrically induced relaxations (Takeda *et al.*, 1995; Hedlund *et al.*, 1997). Thus, nitric oxide has been postulated to play a significant role in regulating prostate smooth muscle relaxation through stimulation of cGMP production (Figure 4) and, in turn, activation of K_{ATP} channels (Cook *et al.*, 2002). Targeting this pathway has been investigated in clinical trials which show that lower urinary tract symptoms associated with BPH improve under the influence of agents that enhance nitric oxide activity such as direct nitric oxide donors (Klotz *et al.*, 1999) or PDE5 inhibitors such as sildenafil (Sairam *et al.*, 2002).

Novel targets: interstitial pacemaker cells

Spontaneous contractions in the guinea pig prostate have been reported to be associated with the firing of 'slow waves' recorded in the smooth muscle stroma using electrophysiological techniques (Exintaris *et al.*, 2002). This slow wave activity is myogenic in origin as it is unaffected by blockers of neural propagation or transmission, such as tetrodotoxin, guanethidine, atropine, capsaicin or indomethacin (Exintaris *et al.*, 2002). Further characterization of this spontaneous electrical activity showed that it is dependent on an external source of Ca^{2+} (Exintaris *et al.*, 2002). More recently, it was established that slow waves recorded in the guinea pig prostate are also dependent on the cycling of Ca^{2+} from inositol triphosphate-dependent Ca^{2+} stores and the buffering of Ca^{2+} by mitochondria (Exintaris *et al.*, 2009). In contrast, voltage-activated 4-amino-pyridine-sensitive K^+ channels, as well as iberiotoxin-sensitive large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels regulate the time-course and frequency of the spontaneous electrical events as demonstrated by patch-clamping freshly digested guinea pig prostatic cells (Oh *et al.*, 2003; Lang *et al.*, 2004) or human prostatic smooth muscle cells (Sui *et al.*, 2004).

A discrete population of prostatic interstitial cells is thought to generate this electrical activity. In the guinea pig prostate gland, these cells lie between the glandular and stromal smooth muscle layers of the individual acini (Exintaris *et al.*, 2002). Similar cells have been previously described in the rat prostate (Aumuller *et al.*, 1987) and more recently in the human prostate (Van der Aa *et al.*, 2003; Shafik *et al.*, 2005). Electron microscopic examination has revealed that these prostatic interstitial cells lack myosin filaments, possess many mitochondria and have an incomplete basal lamina. In contrast, prostatic smooth muscle cells comprise a continuous basal lamina, relatively few mitochondria and an abundance of contractile filaments (Exintaris *et al.*, 2002). By analogy with the gut, it is likely that prostatic interstitial cells provide the depolarizing pulse to neighbouring smooth muscle cells, initiating slow wave activity and subsequent contractility. It is also likely that prostatic interstitial cells are modulated by intrinsic nerves (Exintaris *et al.*, 2006; Dey *et al.*, 2009).

It is conceivable that pathological changes to prostatic interstitial cells or disruption to prostatic interstitial cell/

nerve/smooth muscle networks may play a role in disorders of the prostate gland (Exintaris *et al.*, 2006). An understanding of the cellular mechanisms underlying the prostatic rhythmic contractile effects of nerve-mediated agents could lead to the development of therapeutic agents to treat prostate-specific conditions such as BPH. However, whether a drug can be tailored to target this type of cell is yet to be elucidated.

Concluding remarks

It would be highly desirable to have a BPH therapy that affects the progression of disease rather than only symptom relief. Nevertheless, drugs which relax prostatic smooth muscle are faster acting, more effective and better tolerated than those which shrink or reduce prostate volume when it comes to treating BPH (Hutchison *et al.*, 2007). Furthermore, there is poor correlation between the severity of lower urinary tract symptoms caused by BPH and prostate size (Eckhardt *et al.*, 2001a,b,c). Thus, while an effective agent for BPH that affects disease progression remains elusive, it would seem that the best strategy to improve drugs used in the treatment of BPH would be to concentrate on finding mechanisms which can produce prostatic relaxation greater than that produced by the currently available α_1-adrenoceptor antagonists.

α_1-Adrenoceptor blockade is only able to block approximately half of the contractile response to nerve stimulation in most species studied (Table 3). This implies that inhibiting the nonadrenergic residual response may provide a target, which when used in combination with α_1-adrenoceptor antagonists will provide greater relaxation of prostatic smooth muscle and therefore, greater relief from symptoms. In moderate to severe cases, improved efficacy of drugs may be sufficient for some patients to avoid the need for surgery. Of the possible contenders described in this review, and summarized in Figures 3 and 4, as well as other possible targets not listed here, it would appear that muscarinic receptor antagonists are the most likely candidates. Among all the rest, PDE inhibitors, nitric oxide donors and perhaps $P2X_1$-purinoceptor antagonists also offer some hope.

Conflict of interest

The authors state no conflicts of interest.

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