British Journal of Pharmacology (2006) 147, S88–S119
www.nature.com/bjp

α_1-, α_2- and β-adrenoceptors in the urinary bladder, urethra and prostate

*,[1]Martin C. Michel & [1]Wim Vrydag

[1]Department of Pharmacology & Pharmacotherapy, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands

1 We have systematically reviewed the presence, functional responses and regulation of α_1-, α_2- and β-adrenoceptors in the bladder, urethra and prostate, with special emphasis on human tissues and receptor subtypes.

2 α_1-Adrenoceptors are only poorly expressed and play a limited functional role in the detrusor. α_1-Adrenoceptors, particularly their α_{1A}-subtype, show a more pronounced expression and promote contraction of the bladder neck, urethra and prostate to enhance bladder outlet resistance, particularly in elderly men with enlarged prostates. α_1-Adrenoceptor agonists are important in the treatment of symptoms of benign prostatic hyperplasia, but their beneficial effects may involve receptors within and outside the prostate.

3 α_2-Adrenoceptors, mainly their α_{2A}-subtype, are expressed in bladder, urethra and prostate. They mediate pre-junctional inhibition of neurotransmitter release and also a weak contractile effect in the urethra of some species, but not humans. Their overall post-junctional function in the lower urinary tract remains largely unclear.

4 β-Adrenoceptors mediate relaxation of smooth muscle in the bladder, urethra and prostate. The available tools have limited the unequivocal identification of receptor subtypes at the protein and functional levels, but it appears that the β_3- and β_2-subtypes are important in the human bladder and urethra, respectively. β_3-Adrenoceptor agonists are promising drug candidates for the treatment of the overactive bladder.

5 We propose that the overall function of adrenoceptors in the lower urinary tract is to promote urinary continence. Further elucidation of the functional roles of their subtypes will help a better understanding of voiding dysfunction and its treatment.
British Journal of Pharmacology (2006) **147,** S88–S119. doi:10.1038/sj.bjp.0706619

Keywords: Bladder; urethra; prostate; α_1-adrenoceptor; α_2-adrenoceptor; β-adrenoceptor

Abbreviations: BPH, benign prostatic hyperplasia; OAB, overactive bladder; RNase, ribonuclease; RT–PCR, reverse transcriptase–polymerase chain reaction

Introduction

The lower urinary tract is responsible for urine storage and voiding (see Andersson & Wein, 2004). During the storage phase of the micturition cycle, the bladder relaxes to accommodate increasing volumes of urine at acceptable pressure, and the bladder neck and urethra contract to provide resistance to prevent involuntary leakage. During the micturition phase, the bladder neck and urethral muscles relax to allow the detrusor to contract and expel urine without major resistance. While the prostate does not appear to play a major physiological role in continence, its enlargement in patients with benign prostatic hyperplasia (BPH) can increase bladder outlet resistance and thereby disturb physiological voiding.

Diseases of the lower urinary tract are frequent in the general population. They include the syndrome of the overactive bladder (OAB), which is defined as urgency, with or without incontinence, usually accompanied by frequency and nocturia (Abrams *et al*., 2002), and is present in about 16% of the population aged 40 years and over (Milsom *et al*., 2001). Another frequent condition is stress urinary incontinence, a condition largely affecting the female population. Its reported prevalence in the general female population ranges between 5 and 37% (Hampel *et al*., 2004). The reasons for this remarkable heterogeneity include differences between study populations and the use of varying definitions of the condition. More consistently, stress incontinence accounts for approximately 80% of incontinence in women (Hampel *et al*., 2004). While BPH is a very frequent condition in elderly males, its prevalence estimates depend on whether the histological diagnosis of BPH or the associated bothersome symptoms are assessed, the latter being reported in about 30% of men aged 50–80 in population-based studies (Berges *et al*., 2001).

The autonomic nervous system plays a key role in the regulation of lower urinary tract function (see Bannowsky & Juenemann, 2003; Michel *et al*., 2005c). Its sympathetic innervation occurs *via* the hypogastric nerve arising from the *nucleus intermediolateralis* of spinal cord segments Th_{12}–L_2. Noradrenaline released from these nerves can act on all three

*Author for correspondence at: Department of Pharmacology & Pharmacotherapy, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands; E-mail: m.c.michel@amc.uva.nl

classes of adrenoceptors, that is, α_1-, α_2- and β-adrenoceptors. Three receptor subtypes have been cloned within each of these classes and are designated as α_{1A} (in earlier papers, sometimes also referred to as $\alpha_{1A/D}$ or α_{1C}), α_{1B}, α_{1D}, α_{2A} (its rodent analogue sometimes referred to as α_{2D}), α_{2B}, α_{2C}, β_1, β_2 and β_3 (see Bylund *et al*., 1994; Hieble *et al*., 1995). Multiple splice variants of the α_{1A}-adrenoceptor have been reported, but they have a very similar ligand recognition profile and hence their pharmacological relevance remains unclear (Hirasawa *et al*., 1995; Chang *et al*., 1998; Daniels *et al*., 1999). Moreover, the α_{1A}-adrenoceptor gene product can exhibit low affinity for prazosin and several other drugs upon expression in some cell types, and this phenotype is often referred to as 'α_{1L}' (Ford *et al*., 1997; Daniels *et al*., 1999). Similarly, the β_1-adrenoceptor gene product can exhibit low affinity for propranolol and several other drugs upon expression in some cell types, and this phenotype is sometimes referred to as 'β_4' and sometimes as 'atypical β-adrenoceptor' (Joseph *et al*., 2003; 2004). Moreover, it should be considered that single-nucleotide polymorphisms exist for most of the nine cloned human adrenoceptor subtypes, which could lead to altered tissue responses (see Leineweber *et al*., 2004; Lei *et al*., 2005). The present manuscript reviews the expression, functional responses and regulation of each of these adrenoceptor subtypes in the bladder, urethra and prostate, and discusses their therapeutic implications and potential value as drug targets.

Bladder

The bladder stores and expels urine. The force needed to expel it during the voiding phase of the micturition cycle is generated by the detrusor smooth muscle (sometimes with the help of increasing intra-abdominal pressure), which is anatomically largely found in the bladder dome. In contrast, the bladder neck is involved in generating resistance during the filling phase of the micturition cycle to help prevent involuntary urine leakage. Therefore, the bladder neck is functionally more closely related to the urethra than to the detrusor. The trigone and bladder base are anatomically located close to the bladder neck. Interestingly, the bladder neck appears to have a much denser sympathetic innervation than the detrusor, and the role of neuronally released noradrenaline in activating adrenoceptors expressed in the detrusor has not been well established.

α_1-Adrenoceptors

mRNA expression The presence of α_1-adrenoceptor subtype mRNA in the urinary bladder has been assessed in rats, mice, monkeys and humans, with rats and humans apparently differing considerably. Using competitive RT–PCR, α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors were found to account for 95, 1 and 4%, respectively, of total α_1-adrenoceptor mRNA in rat bladder (Scofield *et al*., 1995). Another study based upon RNase protection assays has reported a roughly similar abundance of all three subtypes in whole rat bladder, but did not provide quantitative information (Malloy *et al*., 1998), whereas a later, more quantitative report from those investigators based upon competitive RT–PCR has shown the presence of the three subtypes in a ratio of 70 : 5 : 25% (Hampel *et al*., 2002). Microarray analysis detected hybridization signals for the α_{1A}-adrenoceptor, but not for any other α-adrenoceptor subtype (Lluel *et al*., 2003b). For each of the three subtypes, expression in the rat bladder base was shown to be markedly greater than in the detrusor (Yono *et al*., 2004). A predominant expression of the α_{1A}-subtype was qualitatively confirmed using *in situ* hybridization studies, which found a strong expression of this subtype in the urothelium, a moderate expression in smooth muscle (quantitatively similar to that in prostate smooth muscle), but no presence in connective tissue; bladder dome and bladder base were similar in this regard (Walden *et al*., 1997). The same study also found a very similar situation in the rhesus monkey bladder (Walden *et al*., 1997). In contrast, that study detected a moderate expression of α_{1A}-adrenoceptors in the human bladder dome smooth muscle (quantitatively similar to that in prostate smooth muscle), but not in bladder dome connective tissue or urothelium or in prostate epithelium (Walden *et al*., 1997). Using real-time PCR, other investigators confirmed a moderate expression of α_1-adrenoceptor mRNA in the human bladder (corresponding to only 3% of β-adrenoceptor mRNA abundance), to which α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors contributed 33, 53 and 14%, respectively (Nomiya & Yamaguchi, 2003). RT–PCR studies reported a dominant abundance of α_{1A}- and α_{1D}-adrenoceptor mRNA with less, if any, α_{1B}-adrenoceptor mRNA in the human bladder (Sigala *et al*., 2004). Other investigators, using RNase protection assays, detected α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors in a 34 : 0 : 66% ratio (Malloy *et al*., 1998). The predominance of α_{1D}-adrenoceptor mRNA has been confirmed in a recent study from the same group using two independent sets of samples using quantitative real-time PCR confirmed by RNase protection assays (Schwinn, personal communication). Real-time PCR studies in mice reported α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors in a 42 : 8 : 50% ratio (Chen *et al*., 2005), that is, in a roughly similar ratio as in the human bladder. Taken together, the total quantity of α_1-adrenoceptor mRNA expression in the detrusor appears low. While the α_{1A}-adrenoceptor is the most abundant subtype in the rat bladder, the relative contributions of α_1-adrenoceptor subtypes in the human bladder remain controversial.

Protein expression The presence of α_1-adrenoceptors in the detrusor of rats, rabbits, guinea-pigs, pigs, cats, monkeys and humans has been examined at the protein level using radioligand-binding studies in tissue homogenates and, in some cases, receptor autoradiography. Using [^{125}I]BE 2254 (also known as [^{125}I]HEAT) as the radioligand, a low density of α_1-adrenoceptors ($\approx$7 fmol mg^{-1} protein) was found in rat bladder, which was shown to represent a homogeneous population of α_{1A}-adrenoceptors (Hampel *et al*., 2002). A low density of α_1-adrenoceptors was confirmed in autoradiography studies using [^{3}H]prazosin (Monneron *et al*., 2000). Saturation-binding experiments with the α_{1A}-selective radioligand [^{3}H]L-771,688 also detected a relatively low density of this subtype in the rat bladder as compared to several other tissues (Chang *et al*., 2000). In rabbit bladder, a slightly greater but still only moderate α_1-adrenoceptor density (14–18 fmol mg^{-1} protein) was reported using [^{125}I]BE 2254 (Tsujimoto *et al*., 1986) or [^{3}H]prazosin (Latifpour *et al*., 1990). Autoradiography studies using [^{3}H]prazosin also detected only few, if any, α_1-adrenoceptors in the guinea-pig, cat and female pig bladder (Monneron *et al*., 2000). Using the same radioligand in membrane preparations, no quantifiable

amounts of α_1-adrenoceptors were detected in male or female porcine detrusor (Goepel *et al*., 1997). Autoradiography studies with [^{3}H]prazosin found very little α_1-adrenoceptor expression at the protein level in the urothelial or smooth muscle layers of the rhesus monkey detrusor (Walden *et al*., 1997). Studies in the human detrusor using [^{125}I]BE 2254 as the radioligand reported a low α_1-adrenoceptor density ($\approx$6 fmol mg^{-1} protein); based upon competition studies with BMY 7378, 66% of these were described as α_{1D}-adrenoceptors (Malloy *et al*., 1998). A low level of α_1-adrenoceptor expression at the protein level in the detrusor was confirmed using [^{125}I]BE 2254 by other investigators (Sigala *et al*., 2004). Using Western blots with subtype-selective antibodies, the latter study demonstrated the presence of all three subtypes at the protein level in the human detrusor, but did not provide subtype-specific quantification (Sigala *et al*., 2004). Another group, however, using [^{3}H]prazosin as the radioligand, has not detected quantifiable numbers of α_1-adrenoceptors in the human detrusor (Goepel *et al*., 1997). Thus, the overall density of α_1-adrenoceptors in the detrusor of various species, including humans, is low.

The presence of α_1-adrenoceptors has also been investigated in the trigone, bladder base and/or bladder neck of several species. In this regard, pigs appear to be the only species where α_1-adrenoceptors have not been detected in the bladder neck (Goepel *et al*., 1997). In the rabbit bladder base, early studies had found α_1-adrenoceptors of an unspecified subtype (Andersson *et al*., 1984; Larsson *et al*., 1986; Levin *et al*., 1988). Direct comparative studies in rats (Monneron *et al*., 2000) and rabbits (Latifpour *et al*., 1990) reported greater α_1-adrenoceptor binding in the trigone and bladder base, respectively, than in the dome. Similar autoradiography studies with [^{3}H]prazosin found greater α_1-adrenoceptor expression in the monkey bladder base than detrusor; based upon competition by the highly α_{1A}-selective SNAP 5272, the latter appeared to predominantly represent α_{1A}-adrenoceptors (Figure 1) (Walden *et al*., 1997). α_1-Adrenoceptors have also been found in the human bladder base (Levin *et al*., 1988). This was confirmed by other investigators, using not only radioligand binding but also Western blotting with subtype-selective antibodies, which detected all three subtypes (Sigala *et al*., 2004). Thus, in agreement with the mRNA measurements, radioligand binding and receptor autoradiography studies have detected only low densities of α_1-adrenoceptors in the detrusor of several species, including humans; in this regard, detection by [^{125}I]BE 2254 appears to be more sensitive than that by [^{3}H]prazosin. A more consistent, and in some direct comparative studies greater, α_1-adrenoceptor expression was seen in the trigone/bladder base/bladder neck region. While the α_{1A}-adrenoceptor appears to be the most abundant subtype in healthy rats, the α_{1D}-adrenoceptor appears to be the most abundant subtype in humans.

In vitro *function* *In vitro* studies on the functional role of α_1-adrenoceptors in the urinary bladder have focused not only on direct contractile effects but also on the modulation of neurotransmitter release. The α_1-adrenoceptor agonists phenylephrine and methoxamine enhanced the field stimulation-induced release of both noradrenaline and acetylcholine in the isolated rat bladder (Somogyi *et al*., 1995). Phenylephrine also increased the basal release of noradrenaline, but not of acetylcholine. While the phenylephrine effect on acetylcholine release was blocked by the α_1-adrenoceptor antagonist terazosin, that on noradrenaline release was not, indicating

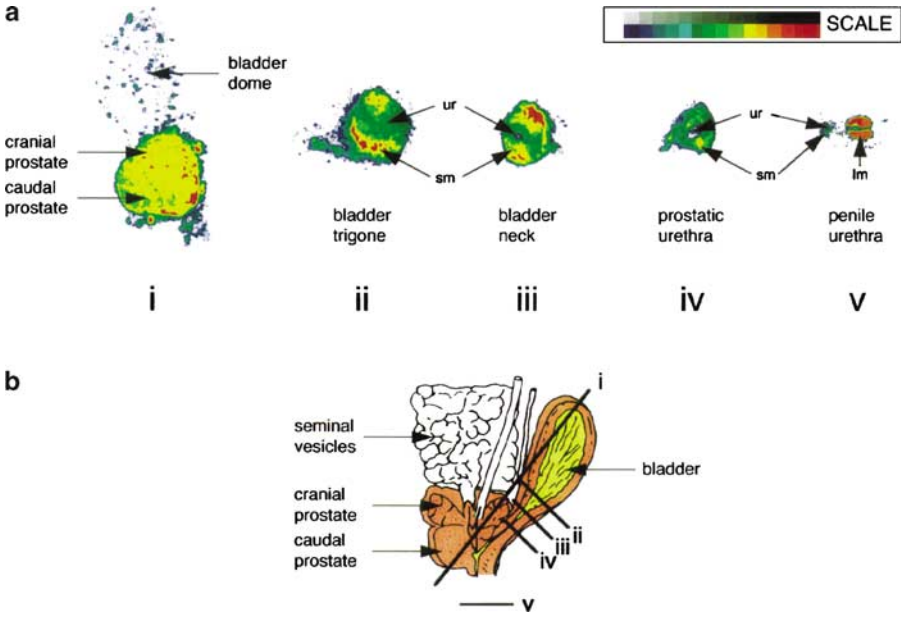

Figure 1 Presence of α_{1A}-adrenoceptor protein in the lower urinary tract of the monkey. Receptors were localized by autoradiography, using [^{3}H]praozsin and defining non-specific binding in the presence of SNAP 5272. Receptor autoradiograms were scanned into computer as a 16 grey scale image. The 16 grey levels corresponding to specific α_{1A}-adrenoceptor receptor binding were each assigned colour (see scale) to allow subtle differences in film exposure to be easily visible. Sections show bladder dome and prostate (i), bladder trigone (ii), bladder base (iii), prostatic urethra (iv) and penile urethra (v). Key: sm (smooth muscle); ur (urothelium); lm (longitudinal muscle). Schematic representation of monkey urinary tract together with the orientation of sectioning planes (i)–(v) shown in (b). Taken with permission from Walden *et al*. (1997).

that the latter may have been β-adrenoceptor-mediated. The increased acetylcholine release was accompanied by an enhancement of field stimulation-induced contraction, which was mainly seen at low-frequency nerve stimulation and at low extracellular Ca^{2+} concentrations. It was concluded that cholinergic nerve terminals in rat bladder express α_1-adrenoceptors that facilitate acetylcholine release. Later studies from these investigators confirmed the initial observations and further demonstrated that the facilitation of acetylcholine release was largely, if not exclusively, mediated by α_{1A}-adrenoceptors (Szell *et al*., 2000). An α_1-adrenoceptor-mediated depolarization of parasympathetic nerves in the vesical ganglia has also been demonstrated in cats (Nakamura *et al*., 1984).

The possible direct contractile effects of α_1-adrenoceptor stimulation have been investigated in rat, rabbit, guinea-pig and human bladder. In the rat detrusor, α_1-adrenoceptor agonists such as phenylephrine produced only weak contractions, that is, in the range of 10–43% of those reached by muscarinic receptor stimulation or receptor-independently by KCl (Kolta *et al*., 1984; Somogyi *et al*., 1995; Lluel *et al*., 2000; 2003a, b; Szell *et al*., 2000). The effect appears to be mediated by a subtype with low affinity for the α_{1D}-selective BMY 7378, most likely the α_{1A}-adrenoceptor (Lluel *et al*., 2003b). Interestingly, and in line with the data on α_1-adrenoceptor expression at the protein level (see above), the phenylephrine-induced contraction was about three times as large in rat bladder neck as compared to the detrusor (Lluel *et al*., 2003a). One study demonstrates that the weak direct contractile effects of α_1-adrenoceptor agonists in the rat detrusor occur *via* chloroethylclonidine-sensitive α_{1B}- or α_{1D}-adrenoceptors, that is, a different subtype than the one mediating enhanced acetylcholine release and hence indirect contractile effects (Szell *et al*., 2000). The direct contractile effects of α_1-adrenoceptor agonists in the rabbit detrusor were also weak (Ueda *et al*., 1984; Tsujimoto *et al*., 1986; Latifpour *et al*., 1990). However, several direct comparative studies demonstrate greater α_1-adrenoceptor-mediated contraction in rabbit trigone (Ueda *et al*., 1984) and bladder base (Latifpour *et al*., 1990). Interestingly, some data show that the α_1-adrenoceptor subtype mediating the contraction of rabbit trigone, bladder base and/or bladder neck resembles the cloned α_{1A}-adrenoceptor (Honda & Nakagawa, 1986; van der Graaf *et al*., 1997; Kava *et al*., 1998; Williams *et al*., 1999), but has relatively low affinity for prazosin (pA_2 8.0–8.4), indicating that it may belong to the α_{1L}-phenotype of the α_{1A}-adrenoceptor (Lefevre-Borg *et al*., 1993; Deplanne & Galzin, 1996; van der Graaf *et al*., 1997; Kava *et al*., 1998; Williams *et al*., 1999) (a more detailed discussion of the α_{1L}-phenotype is given in the prostate section). Based upon the high potency of the antagonist L-771,688 (also known as SNAP 6383) in inhibiting the contractile effects of the agonist A61603, the receptor mediating contraction of the monkey bladder neck was also classified as being α_{1A} (Chang *et al*., 2000).

In analogy to rats and rabbits, studies in the human detrusor have found only very weak contraction (up to 5% of the maximum muscarinic response) by the α_1-adrenoceptor agonist phenylephrine (Nomiya & Yamaguchi, 2003). A more robust contraction was observed in studies with the human bladder base and bladder neck (Caine *et al*., 1975). In the latter tissue, contraction was potently elicited by the α_{1A}-selective agonist A-61603 and inhibited potently by the α_{1A}-selective antagonist L-771,688 (Chang *et al*., 2000). In contrast to the rabbit bladder neck, however, responses in the human bladder base exhibited high potency for prazosin (pA_2 8.9) (Kunisawa *et al*., 1985), indicating that the α_{1L}-phenotype of the α_{1A}-adrenoceptor was not involved.

In contrast to rats, rabbits and humans, α_1-adrenoceptor stimulation in the isolate guinea-pig bladder did not enhance, but rather inhibited, the amplitude and frequency of phasic contractions (Gillespie, 2004), but the reasons for such species differences remain unclear. Thus, in most species, including humans, α_1-adrenoceptor stimulation produces only weak detrusor contraction, whereas a stronger contraction is observed for the trigone, bladder base and/or bladder neck. The physiological relevance of this, however, remains unclear, since the bladder neck appears largely under the control of the parasympathetic (and perhaps nonadrenergic–noncholinergic) rather than the sympathetic nervous system (Deplanne *et al*., 1998).

In vivo *function* The *in vivo* analysis of a role for α_1-adrenoceptors in the regulation of bladder function is complicated by the fact that both central and peripheral receptors may be involved and may serve distinct functions. In anaesthetized rats, intra-thecal injections of prazosin inhibited bladder contraction evoked from the locus coeruleus (Yoshimura *et al*., 1988). Using continuous cystometry in conscious rats, doxazosin given intra-thecally was shown to reduce the size of the bladder pressure (Ishizuka *et al*., 1996b). Two studies have investigated the α_1-adrenoceptor subtypes involved in the central stimulation of bladder contraction. Reductions in the height of isovolumetric contraction were reported for the α_{1A}-adrenoceptor antagonist RS 100,329 given intra-thecally and for the moderately α_{1B}-selective antagonist (+)-cyclazosin; however, the latter effect was not dose-related (Yoshiyama & De Groat, 2001). Further, both drugs increased the frequency of these contractions, while the α_{1D}-adrenoceptor antagonist BMY 7378 had no effect. Naftopidil, which may have some selectivity for α_{1D}-adrenoceptors, given intra-thecally, was reported to inhibit the appearance of regular isovolumetric bladder contractions and reduce their height (Sugaya *et al*., 2002). In addition, tamsulosin, which has high affinity for both α_{1A}- and α_{1D}-adrenoceptors, was also reported to inhibit the appearance of these contractions. These studies demonstrate that central, most likely spinal, α_1-adrenoceptors are involved in stimulating bladder contractility, and that an α_{1A}-adrenoceptor is the most likely candidate mediating such effects.

Studies with systemic administration of α_1-adrenoceptor antagonists have yielded less consistent results. For example, i.v. α_1-adrenoceptor antagonists inhibited the sympathetic control of the bladder by reducing hypogastric nerve activity (Danuser & Thor, 1995; Ramage & Wyllie, 1995) and somatic activity to the urethra (Danuser & Thor, 1995). However, spontaneous bladder contractions, presumably mediated by the parasympathetic nervous system, were unaffected (Ramage & Wyllie, 1995). Others compared the intra-thecal and intra-arterial effects of doxazosin, phentolamine, prazosin, tamsulosin and yohimbine upon cystometric parameters in anaesthetized rats (Jeong & Lee, 2000); based upon differences between drugs and modes of administration, these authors proposed that α_1-adrenoceptors suppress the micturition effect *via* a peripheral mechanism, whereas α_2-adrenoceptors

do so *via* a central mechanism. Finally, antagonists selective for α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors, that is, RS-100,329, RS-51,385 and BMY 7378, given i.v. to anaesthetized rats, were found to have no effect on bladder contraction height induced by infusion of saline into the bladder, but the associated reflex urethral contractions were attenuated by blockade of $\alpha_{1A/D}$-adrenoceptors (Conley *et al*., 2001). Both RS-100,329 and BMY 7378 also decreased resting urethral pressure. The failure to see any changes in evoked bladder contraction may reflect a difference in the method being used to evoke it and/or the route of administration. However, the increase in frequency observed for intra-thecal α_{1A}-adrenoceptor antagonists would be expected to be translated into a decrease in the volume threshold, but this was also not observed.

Thus, despite some ongoing controversies, the overall *in vivo* data suggest that at a spinal level α_1-adrenoceptors are probably involved in mediating bladder contractions and decreasing the frequency of micturition. Therefore, systemically administered α_1-adrenoceptor antagonists that penetrate into the central nervous system may predominantly inhibit bladder contractions. On the other hand, α_1-adrenoceptors are also involved in the peripheral control of the sympathetic supply to the bladder and thus storage. In this respect, stimulation of the hypogastric nerve has also been shown to facilitate cholinergic transmission at the level of the pelvic ganglia *via* the action of α_1-adrenoceptors (Keast *et al*., 1990) and thus also enhancing bladder contractions. Interestingly, it has been reported that in anaesthetized dogs the α-adrenoceptor agonist midodrine did not affect bladder capacity in young animals, but reduced it in old animals (Takahashi *et al*., 1996).

Treatment with a very high dose of the α_1-adrenoceptor antagonist doxazosin ($30\,mg\,kg^{-1}$ orally) was reported to attenuate obstruction-induced bladder hypertrophy (Das *et al*., 2002). However, these findings are difficult to interpret since another study in nonobstructed rats found that doxazosin (2 or $4\,mg\,kg^{-1}$ s.c. plus $4\,mg\,kg^{-1}$ orally) increased the weight of the bladder base and, in at least some dose groups, upregulated α_{1A}-adrenoceptor mRNA in the bladder base (Yono *et al*., 2004), and also because the doxazosin doses in both studies are very high as compared to a therapeutic dose of 4–8 mg per patient. Such high doses of doxazosin may have growth-inhibiting or apoptotic effects on the lower urinary tract, which are independent of α_1-adrenoceptors (Walden *et al*., 2004).

Regulation of receptor expression and function Some studies have investigated a possible regulation of the role of α_1-adrenoceptors in bladder function by gender, ageing and bladder outlet obstruction. Expression of α_{1A}-adrenoceptor mRNA was similar in the detrusor and bladder base of male and female rats (Walden *et al*., 1997), and the number of α_1-adrenoceptor-binding sites was also similar in the detrusor and bladder base of male and female rabbits (Latifpour *et al*., 1990). A study in humans confirmed a lack of gender effect on α_1-adrenoceptor binding in detrusor and bladder neck, but found a significantly greater α_1-adrenoceptor density in female than in male trigone; this study also reported on the quantity of α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor mRNA in all three regions of both genders, but did not provide a statistical analysis of the observed differences (Sigala *et al*., 2004).

Ageing studies on α_1-adrenoceptors in the bladder have been reported for rats, rabbits and dogs. A comparison of 7-, 17- and 29-month-old Fischer rats did not detect significant alterations in the maximum effects or potency of phenylephrine (Kolta *et al*., 1984). This was confirmed in studies on 10- and 30-month-old female (Lluel *et al*., 2000) and male Wistar rats (Lluel *et al*., 2003b), as well as in 6- and 24-month-old male Sprague–Dawley rats (Lluel *et al*., 2003a). However, the former two studies surprisingly reported a markedly increased noradrenaline-induced contraction in aged animals, which was not explained by a possible α_2-adrenoceptor stimulation (Lluel *et al*., 2000; 2003b). Since the same studies did not detect differential expression of any α-adrenoceptor subtype in a microarray analysis, the phenylephrine findings appear somewhat more plausible than the noradrenaline findings. Ageing was also reported not to affect the number of α_1-adrenoceptor-binding sites in detrusor and bladder base of 6 months vs 4.5–5-year-old male or female rabbits (Latifpour *et al*., 1990). On the other hand, studies in anaesthetized dogs found reductions of bladder capacity upon systemic administration of the α_1-adrenoceptor agonists in 68-month-old, but not in 12-month-old, animals; the interpretation of these findings, however, is complicated by the fact that the old dogs had been parous, whereas the young ones were nonparous (Takahashi *et al*., 1996). Thus, the overall data suggest that neither gender nor ageing has a major effect on α_1-adrenoceptor function in the bladder.

Due to the high prevalence of BPH, bladder outlet obstruction is frequent in elderly men. Since it has been speculated that the α_1-adrenoceptor antagonist-induced symptom relief in BPH patients may involve effects on bladder function (see Michel, 2002; Roehrborn & Schwinn, 2004), potential alterations of α_1-adrenoceptors have been investigated in the bladder of animal models of obstruction and in patients. Studies in a rat model of obstruction found an unchanged total α_1-adrenoceptor mRNA and radioligand binding. However, this was accompanied by a reduction of α_{1A}- and an increase of α_{1D}-adrenoceptor mRNA; in competition-binding experiments, α_{1D}-adrenoceptors had been undetectable in control rats, but represented approximately 40% of all α_1-adrenoceptors in obstructed animals (Hampel *et al*., 2002). The contractile effects of phenylephrine were reported to remain unchanged in obstructed patients (Nomiya & Yamaguchi, 2003). Further studies are needed to define the role of α_1-adrenoceptors in the detrusor in settings of bladder outlet obstruction.

Clinical implications In conclusion, α_1-adrenoceptors appear to play a small functional role if any in the detrusor of healthy animals and humans. Since some of the beneficial effects of α_1-adrenoceptor antagonists in BPH patients cannot easily be explained solely based upon prostatic α_1-adrenoceptors (see the section on prostate), it nevertheless appears plausible that those located in the detrusor may have therapeutic relevance. This hypothesis, however, remains to be tested. In a similar vein, it has been reported in a small group of patients with spinal cord injury that treatment with the α_1-adrenoceptor antagonist terazosin increases bladder compliance and results in less incontinence and dysreflexia (Swierzewski *et al*., 1994), but it remains unclear whether this reflects a direct effect on the bladder or an indirect effect. On the other hand, α_1-adrenoceptors may play a more

prominent functional role in the bladder neck and hence the regulation of bladder outlet resistance. Antagonizing their function may contribute to the beneficial effects of α_1-adrenoceptor antagonists in BPH patients (see the section on prostate), whereas their stimulation provides a potential target in the treatment of stress incontinence (see the section on urethra).

α_2-*Adrenoceptors*

mRNA and protein expression To the best of our knowledge, the presence of α_2-adrenoceptor subtype mRNA in the bladder has not been reported. At the protein level, however, radioligand-binding studies have detected α_2-adrenoceptors in the detrusor and bladder base/bladder neck of rabbits (Andersson *et al*., 1984; Levin *et al*., 1988; Latifpour *et al*., 1990), pigs (Goepel *et al*., 1997) and humans (Levin *et al*., 1988; Goepel *et al*., 1997). Their density in the rabbit bladder base was reported to be smaller (Levin *et al*., 1988), larger (Andersson *et al*., 1984) and similar (Latifpour *et al*., 1990) to that of α_1-adrenoceptors within the same tissue. In the porcine and human bladder, their density (15–25 and 40 fmol mg^{-1} protein, respectively) clearly exceeded that of α_1-adrenoceptors, but was somewhat smaller than that of β-adrenoceptors within the same study (Goepel *et al*., 1997). In competition-binding experiments in porcine and human bladder, a predominant, if not exclusive, population of α_{2A}-adrenoceptors was found (Goepel *et al*., 1997).

In vitro *function* Only few studies have assessed the functional role of α_2-adrenoceptors in the bladder. The prejunctional inhibition of neurotransmitter release from both post-ganglionic sympathetic and parasympathetic nerve terminals is the best-established function of α_2-adrenoceptors in most tissues. Consistent with this concept, α_2-adrenoceptor stimulation inhibited field stimulation-induced contraction of rat bladder in a tetrodotoxin, but not hexamethonium-sensitive, manner (Santicioli *et al*., 1983; Maggi *et al*., 1985). Moreover, α_2-adrenoceptor stimulation also inhibits parasympathetic nerve activity in the bladder of rabbits (Tsurusaki *et al*., 1990) and cats (Nakamura *et al*., 1984; Keast *et al*., 1990) by an effect on the vesical parasympathetic ganglion. Despite the considerable abundance of α_2-adrenoceptors in bladder homogenates, their post-junctional function has not been established as they do not mediate contractile effects in the rabbit detrusor (Ueda *et al*., 1984), whole guinea-pig bladder (Gillespie, 2004) or human bladder base (Kunisawa *et al*., 1985).

In vivo *function* *In vivo* studies on α_2-adrenoceptor function in the bladder are often difficult to interpret because central and peripheral α_2-adrenoceptor stimulation may not have the same effects; they may even partly counteract each other and their relative roles may depend on the use of anaesthetized vs conscious animals. In anaesthetized rats α_2-adrenoceptor stimulation reduced volume-induced bladder contraction (Maggi *et al*., 1985; Harada & Constantinou, 1993). Using somewhat different methods, an opposite conclusion was drawn from a more recent study (Jeong & Lee, 2000). However, all of these studies agree that the site of action of the α_2-adrenoceptor agonists and antagonists is in the spinal cord rather than in the periphery. An initial report in conscious rats reported that intra-thecal and intra-arterial (close the bladder) administration of an α_2-adrenoceptor agonist reduced micturition pressure, bladder capacity and micturition volume; while an α_2-antagonist inhibited the effects of intra-thecal agonist, it mimicked those of the peripheral administration (Ishizuka *et al*., 1996a). In contrast, other studies found that α_2-adrenoceptor agonists increase the frequency of bladder contractions (Durant *et al*., 1988; Kontani *et al*., 2000) and voiding (Harada & Constantinou, 1993), but the interpretation of this finding would be complicated by a diuretic effect of the agonist (Harada & Constantinou, 1993). Similar to the situation in anaesthetized rats, all of the above studies agree that α_2-adrenoceptors in the spinal cord are likely to be the main source of modulation of bladder function.

Regulation of receptor expression and function Studies in rabbit detrusor or bladder base reported a similar α_2-adrenoceptor density in male and female animals as well as in young (6 months) and old (4.5–5 years) rabbits (Latifpour *et al*., 1990). A study in male and female pigs confirmed the lack of gender difference in detrusor and bladder neck, with α_{2A}-adrenoceptors being the only detectable subtype in all groups (Goepel *et al*., 1997). Based upon all of the above data, α_2-adrenoceptors are not considered a promising target for the treatment of voiding disorders.

β-*Adrenoceptors*

mRNA expression The presence of β-adrenoceptors in the rat and human bladder at the mRNA level has been studied using Northern blots, PCR and *in situ* hybridization. Messenger RNA for all three β-adrenoceptor subtypes has been detected in rats (Seguchi *et al*., 1998; Fujimura *et al*., 1999; Matsubara *et al*., 2002). It has been claimed that the β_3-adrenoceptor may be the most abundant subtype (Fujimura *et al*., 1999), but no specific quantitative data were reported. Studies in the human bladder have also detected mRNA for all three β-adrenoceptor subtypes (Fujimura *et al*., 1999; Igawa *et al*., 1999; Takeda *et al*., 1999; Li *et al*., 2003; Nomiya & Yamaguchi, 2003). Based upon quantitative PCR experiments, it appears that the β_3-adrenoceptor accounts for more than 95% of all β-adrenoceptor mRNA in the human bladder (Nomiya & Yamaguchi, 2003). The presence of β_3-adrenoceptor mRNA in the human detrusor has also been confirmed in *in situ* hybridization studies (Takeda *et al*., 1999).

Protein expression The identification of β-adrenoceptors at the protein level is typically based upon binding studies with radioligands such as [^{125}I]iodocyanopindolol, [^{125}I]iodopindolol, [^{3}H]CGP 12,177 or [^{3}H]dihydroalprenolol. [^{125}I]iodocyanopindolol and [^{3}H]CGP 12,177 have much lower affinity for β_3- than for β_1- or β_2-adrenoceptors (Hoffmann *et al*., 2004; Baker, 2005). Data from our lab confirm this and further demonstrate that [^{3}H]dihydroalprenolol yields a similarly poor labelling of β_3-adrenoceptors (Niclauss *et al*., unpublished observations), a finding that is entirely consistent with the low β_3-adrenoceptor affinity of unlabelled alprenolol (Hoffmann *et al*., 2004). While high concentrations of [^{125}I]iodocyanopindolol and [^{3}H]CGP 12,177 have successfully been used to label β_3-adrenoceptors in transfected cells, the use of similarly high concentrations in tissues yields very high nonspecific binding and will saturate β_1- and β_2-adrenoceptors. Both problems

make the detection of β_3-adrenoceptors in tissues expressing mixed β-adrenoceptor subtype populations virtually impossible. A potential alternative would be the use of a β_3-adrenoceptor-selective radioligand such as [^{3}H]SB 206,606. However, this ligand has only high nanomolar affinity for β_3-adrenoceptors (K_d values of 200–500 nM) (Muzzin *et al*., 1994; Klaus *et al*., 1995). Therefore, [^{3}H]SB 206,606 is also a poor choice for the labelling of β_3-adrenoceptors in tissues. These technical limitations must be considered when interpreting existing radioligand-binding data in the bladder and other tissues.

Radioligand-binding studies on bladder β-adrenoceptors have been reported for rats, rabbits, pigs and humans. Saturation-binding studies with various radioligands have reported 6–42 fmol mg^{-1} protein in rats (Nishimoto *et al*., 1995; Ma *et al*., 2002), 60–92 fmol mg^{-1} protein in rabbits (Levin *et al*., 1988; Latifpour *et al*., 1990; Morita *et al*., 1998), 30–154 fmol mg^{-1} protein in pigs (Goepel *et al*., 1997; Yamanishi *et al*., 2002b, c) and 22–60 fmol mg^{-1} protein in humans (Levin *et al*., 1988; Goepel *et al*., 1997; Morita *et al*., 2000; Li *et al*., 2003). Limited attempts have been made to identify the receptor subtypes in the bladder by radioligand binding. Based upon competition studies with the β_2-selective antagonist ICI 118,551 and a β_1-selective antagonist, sites in the rabbit (Latifpour *et al*., 1990) and human bladder (Goepel *et al*., 1997) were reported to largely belong to the β_2-subtype. On the other hand, three studies in the porcine bladder detected few, if any, high-affinity sites for ICI 118,551, and the β_1-selective antagonist CGP 20,712A recognized largely low-affinity sites in those studies (Goepel *et al*., 1997; Yamanishi *et al*., 2002b, c). Two of the studies additionally report about 60% high-affinity sites for SR 59,230A (Yamanishi *et al*., 2002b, c); the latter authors interpreted these findings as evidence in favour of the presence of a population of largely β_3-adrenoceptors. However, three reasons argue against this interpretation: Firstly, ICI 118,551 may not be β_2-selective in pigs (Goepel *et al*., 1996), which make the low affinity of this compound in the porcine bladder difficult to interpret. Secondly, while SR 59,230A can be used to functionally block β_3-adrenoceptors, it is not selective for this subtype and, at least in humans, has even slightly lower affinity for β_3- than for β_1- and β_2-adrenoceptors (Hoffmann *et al*., 2004). Thirdly, the radioligands used in all of the above studies are unlikely to label a major fraction of possibly present β_3-adrenoceptors due to their low affinity for this subtype (at least in humans; see above). Therefore, we consider the presently available pig data to be inconclusive. This does not exclude the presence of β_3-adrenoceptors at the protein level in any of these species, but the currently available radioligand-binding techniques are probably inadequate to detect their presence. Hence, the reported densities of β-adrenoceptors in the bladder may represent an underestimation if the additional presence of β_3-adrenoceptors is taken into account.

In vitro *function* Since activation of adenylyl cyclase is the prototypical signalling pathway of β-adrenoceptors, it is not surprising that an isoprenaline-stimulated, propranolol-sensitive elevation of cAMP content has also been reported in rat bladder (Derweesh *et al*., 2000; Ma *et al*., 2002; Uchida *et al*., 2005). However, various recent studies have questioned whether this can sufficiently explain β-adrenoceptor-mediated smooth muscle relaxation (Horinouchi *et al*., 2003; Peters & Michel, 2003; Tanaka *et al*., 2003). One study in rat bladder demonstrated that the concentration–response relationships for isoprenaline, clenbuterol and FR 165,101 for relaxation and cAMP elevations were largely superimposable in noncontracted muscle; however, no such relationship was observed during KCl-induced contraction (Uchida *et al*., 2005). Accordingly, the adenylyl cyclase inhibitor SQ 22,536, in a concentration where it fully suppressed cAMP formation, inhibited rat bladder relaxation by all three agonists in the absence of pre-contraction, but not in its presence (Uchida *et al*., 2005). Similarly, SQ 22,536 and the protein kinase A inhibitors H7, H89 and Rp-cAMPs, if anything, inhibited isoprenaline-induced relaxation of rat bladder only against passive tension, but not against KCl-induced tension in another study (Frazier *et al*., 2005a). These data demonstrate that, at least in rats, elevation of cAMP is relevant for the regulation of bladder smooth muscle tone against passive tension, but not in the presence of a depolarizing stimulus such as KCl. Interestingly, a combination of adenylyl and guanylyl cyclase inhibitors (SQ 22,536 and ODQ) caused the strongest inhibition of relaxation against passive tension, but was also inactive against KCl-induced tension (Frazier *et al*., 2005a).

A possible modulation of membrane potential, ion-channel activity and intracellular ion concentrations has been studied as an alternative means of β-adrenoceptor control of bladder function. In guinea-pig bladder smooth muscle bundles exhibiting spontaneous action potentials, isoprenaline was found to hyperpolarize the cells, prevent action potentials and reduce the associated Ca^{2+} transients; the elevation of membrane potential was blocked by protein kinase A inhibitors and by high extracellular K^+ concentrations, but not by K^+ channel inhibitors (Nakahira *et al*., 2001). In other studies, both isoprenaline and the receptor-independent adenylyl cyclase activator forskolin were shown to increase iberiotoxin-sensitive K^+ currents in guinea-pig bladder smooth muscle cells, and such stimulation was sensitive to a peptidergic inhibitor of protein kinase A (Kobayashi *et al*., 2000). In a later study, these investigators also demonstrated propranolol-sensitive isoprenaline inhibition of Ba^{2+} current through L-type Ca^{2+} channels due to a shift of steady-state for inactivation by 11 mV; this effect was apparently mediated by protein kinase A, but did not involve protein kinase G (Kobayashi *et al*., 2003). Other investigators reported that isoprenaline caused marginal increases in Ca^{2+} currents after large conditioning depolarizations (but not in their absence) in the guinea-pig bladder, and that this effect was not mimicked by forskolin (Smith *et al*., 1999). On the other hand, a third group found that isoprenaline causes intracellular Ca^{2+} sparks and activates voltage-dependent Ca^{2+} channels in guinea-pig bladder, and proposed that this may underlie the activation of large-conductance, iberiotoxin-sensitive K^+ channel (Petkov & Nelson, 2005). Differences in the electrophysiological procedures used by the two groups may have contributed to this apparent controversy. Activation of iberiotoxin-sensitive K^+ channels can relax the urinary bladder (Malysz *et al*., 2004). Several studies have assessed the functional relevance of ion channel modulation by β-adrenoceptor stimulation. Studies using KCl-precontracted bladder strips from guinea-pigs (Kobayashi *et al*., 2000) or rats (Frazier *et al*., 2005a; Uchida *et al*., 2005) have consistently found that K^+ channel blockers such as iberiotoxin or charybdotoxin inhibit isoprenaline-induced bladder relaxation. Interestingly, the

latter two studies also report that relaxation against passive tension is not sensitive to those toxins.

Prostaglandins may play a role in bladder contraction by several agents such as protease-activated receptors or bradykinin (Nakahara *et al*., 2003; 2004; Chopra *et al*., 2005). Therefore, it is surprising that prostaglandins were also postulated to play a permissive role for β-adrenoceptor-mediated relaxation of the urinary bladder (Bolle *et al*., 1999).

The key function of β-adrenoceptors in the bladder is smooth muscle relaxation and an increase in bladder compliance during the filling phase of the micturition cycle. The interpretation of *in vitro* bladder relaxation experiments has to take into account that the results are sensitive to the experimental conditions. Thus, it has been found that the β-adrenoceptor agonist isoprenaline was approximately six times more potent when tested against passive tension than when tested against KCl-induced bladder tone in rats (Frazier *et al*., 2005a; Uchida *et al*., 2005). This is consistent with indirect comparisons in the published literature, where a pEC_{50} for isoprenaline of 8.3 (Yamazaki *et al*., 1998) vs 7.2 (Longhurst & Levendusky, 1999) and of 9.1 (Yamazaki *et al*., 1998) vs 7.3 (Oshita *et al*., 1997) were reported in rats and rabbits, respectively, for passive tension vs pre-contraction. In a comparison between KCl-induced and carbachol-induced tension in rat isolated detrusor, isoprenaline was significantly less potent and effective against the latter (Longhurst & Levendusky, 1999). Moreover, the choice of passive tension vs pre-contraction for relaxation experiments may also affect the underlying signal transduction of the β-adrenoceptor response (Frazier *et al*., 2005a; Uchida *et al*., 2005). A second methodological consideration relates to the use of muscarinic receptor agonists to induce bladder pre-contraction in combination with β-adrenoceptor agonists such as BRL 37,344 to induce relaxation. This drug has affinity for muscarinic acetylcholine receptors in the same concentration range where it acts as a β-adrenoceptor agonist (Kubota *et al*., 2002); hence, data using this combination may at least partly reflect direct muscarinic receptor antagonism rather than β-adrenoceptor agonism (see below).

A relaxation of bladder smooth muscle by β-adrenoceptor agonists has been demonstrated against passive tension (Igawa *et al*., 2001; Takeda *et al*., 2002a), endothelin receptor-mediated (Takeda *et al*., 2003), muscarinic receptor-mediated (Seguchi *et al*., 1998; Nomiya & Yamaguchi, 2003) and KCl-induced pre-contraction (Nishimoto *et al*., 1995; Yamanishi *et al*., 2003a) or against field stimulation-induced tone (Nishimoto *et al*., 1995; Hudman *et al*., 2001). Moreover, relaxation responses have been demonstrated in the detrusor of various species, including rats (Kolta *et al*., 1984; Nishimoto *et al*., 1995; Oshita *et al*., 1997; Seguchi *et al*., 1998; Yamazaki *et al*., 1998; Fujimura *et al*., 1999; Longhurst & Levendusky, 1999; Lluel *et al*., 2000; Morita *et al*., 2000; Woods *et al*., 2001; Matsubara *et al*., 2002; Inci *et al*., 2003; Malysz *et al*., 2004; Uchida *et al*., 2005; Frazier *et al*., 2005a), mouse (Matsui *et al*., 2003), rabbits (Oshita *et al*., 1997; Morita *et al*., 1998; 2000; Yamazaki *et al*., 1998; Bing *et al*., 2003), guinea-pigs (Li *et al*., 1992; Gopalakrishnan *et al*., 1999; Kobayashi *et al*., 2000; Malysz *et al*., 2004), ferrets (Takeda *et al*., 2000a), cats (Nergardh *et al*., 1977), dogs (Yamazaki *et al*., 1998), pigs (Yamanishi *et al*., 2002b, c; 2003a), monkeys (Takeda *et al*., 2002a) and humans (Nergardh *et al*., 1977; Fujimura *et al*., 1999; Igawa *et al*., 1999; 2001; Takeda *et al*., 1999; Morita *et al*., 2000; Nomiya & Yamaguchi, 2003). In contrast, β-adrenoceptor stimulation did not consistently relax the basal tone of the human bladder neck (Caine *et al*., 1975).

Some studies have performed direct inter-species comparisons regarding the ability of β-adrenoceptor agonists to induce bladder relaxation. Such comparisons of, for example, rat vs dog (Takeda *et al*., 2003), rat vs rabbit (Oshita *et al*., 1997) or rat vs rabbit vs dog (Yamazaki *et al*., 1998) have consistently reported that the maximum effects of an agonist without subtype selectivity, such as isoprenaline, were similar in various species. However, within the same study, the rank order of isoprenaline potency consistently was rabbit > rat > dog, suggesting that rabbits may have the largest and dogs the smallest receptor reserve for this response, respectively. Similar inter-species comparisons with subtype-selective β-adrenoceptor agonists are more difficult to interpret, since the subtype being involved may differ between species.

Functional studies into the β-adrenoceptor subtypes mediating bladder relaxation have been hampered by several problems. Firstly, some drugs proposed to be β_3-adrenoceptor-selective agonists may have effects independent of β-adrenoceptors. For example, it was reported that both BRL 37,344 and SR 58,611 can cause vasodilatation, which is insensitive to β-adrenoceptor antagonists (Brahmadevara *et al*., 2003). Moreover, BRL 37,344 was reported to be a direct muscarinic receptor antagonist (Kubota *et al*., 2002) and α_1-adrenoceptor antagonist (Leblais *et al*., 2005). Secondly, no truly β_3-adrenoceptor-selective antagonist has been described. Thus, SR 59,230, the most frequently used drug to antagonize β_3-adrenoceptors, does not discriminate human β-adrenoceptor subtypes (Hoffmann *et al*., 2004) and, similarly to the chemically related bupranolol, may also be an α_1-adrenoceptor antagonist (Leblais *et al*., 2005). When binding to β_3-adrenoceptors, SR 59,230 may exhibit agonist rather than antagonist properties in some tissues (Horinouchi & Koike, 2001). Such limitations should be taken into account when interpreting the functional data presented below.

Studies in various species have used agonist and antagonist potency to identify the functional involvement of β-adrenoceptor subtypes in bladder relaxation. Since absolute agonist potency may differ between species even for nonsubtype-selective agonists (see above), the former approach has used either rank orders of potency of various agonists or the potency of highly subtype-selective agonists to classify the receptor subtype being involved. Most studies have been reported from rats. Based upon a high potency of β_3-selective agonists such as CL 316,243 (Woods *et al*., 2001) and FK175 (Fujimura *et al*., 1999), it has been proposed that rat bladder relaxation predominantly occurs *via* this subtype. However, studies assessing the rank order of potency of multiple subtype-selective agonists have proposed a mixed involvement of β_2- and β_3-adrenoceptors in rat bladder relaxation in most cases. These were based upon rank orders such as isoprenaline = procaterol (β_2-selective) > CL 316,243 > dobutamine (β_1-selective) (Takeda *et al*., 2003), CL 316,243 $\geqslant$ isoprenaline $\geqslant$ procaterol (Takeda *et al*., 2000b), isoprenaline $\geqslant$ CL 316,243 $\geqslant$ procaterol > dobutamine (Yamazaki *et al*., 1998), BRL 37,344 $\geqslant$ isoprenaline (Oshita *et al*., 1997), isoprenaline = GS-332 (β_3-selective) $\geqslant$ clenbuterol (β_2-selective) (Morita *et al*., 2000) or isoprenaline > FR 165101 (β_3-selective) $\geqslant$ clenbuterol $\gg$ dobutamine (Uchida *et al*., 2005). One study, based upon a rank order of agonist potency of isoprenaline > BRL

37,344 ⩾ T-0509 (β_1-selective) > terbutaline (β_2-selective) ⩾ SR 58,611 (β_3-selective), has even proposed a mixed involvement of β_1-, β_2- and β_3-adrenoceptors in rat bladder relaxation (Longhurst & Levendusky, 1999). Antagonist studies have reported that ICI 118,551 inhibits the effects of clenbuterol against low-, but not high-frequency field stimulation (Hudman *et al*., 2000). Relaxant effects of the β_3-agonist FK175 were moderately inhibited by the nonselective bupranolol, but not by even high concentrations of the β_1-selective CGP 20,712 or the β_2-selective ICI 118,551 (Fujimura *et al*., 1999). Similarly, relaxation induced by BRL 37,344 was not inhibited by low propranolol concentrations, but by CGP 12,177 or SR 59,230 when added atop of propranolol; in the same study, relaxation by CGP 12,177 was not affected even by high propranolol concentrations (Longhurst & Levendusky, 1999). These data indicate that β_2- and β_3-selective agonists may indeed cause rat bladder relaxation *via* their cognate receptor subtypes. With regard to nonsubtype-selective agonists such as isoprenaline or noradrenaline, several studies report relatively poor antagonism by propranolol, metoprolol, butoxamine or ICI 118,551 (Oshita *et al*., 1997; Seguchi *et al*., 1998; Longhurst & Levendusky, 1999). However, SR 59,230, which should inhibit the cloned β_3-adrenoceptor, also caused only poor isoprenaline antagonism (Longhurst & Levendusky, 1999). Taken together, these data argue against a strong involvement of β_1- and β_2-adrenoceptors, but also fail to provide clear evidence for a β_3-adrenoceptor. Interestingly, the isoprenaline-induced cAMP response in rat bladder was fully sensitive to propranolol (Ma *et al*., 2002), which is in line with the proposal that β-adrenoceptor-mediated bladder relaxation occurs largely cAMP-independent (Frazier *et al*., 2005a; Uchida *et al*., 2005).

In vitro relaxation studies in rabbit bladder have reported agonist rank orders of potency of isoprenaline ⩾ adrenaline > noradrenaline ⩾ BRL 37,344 (Oshita *et al*., 1997), procaterol > isoprenaline > adrenaline ⩾ CGP 12,177 > noradrenaline ⩾ dobutamine > CL 316,243 (Yamazaki *et al*., 1998) or clenbuterol ⩾⩾ GS-332 (Morita *et al*., 2000). Propranolol, bupranolol and ICI 118,551 antagonized the isoprenaline-induced relaxation with high potency, whereas CGP 20,712, in concentrations up to 100 nM, had no effect (Oshita *et al*., 1997; Yamazaki *et al*., 1998). Taken together, these data demonstrate that relaxation of the rabbit detrusor is predominantly mediated by a β_2-adrenoceptor.

In the porcine detrusor, there was a rank order of potency of salbutamol (β_2-agonist) > noradrenaline > BRL 37,344 > CGP 12,177 (the latter two being partial agonists only); while the BRL 37,344 response was antagonized by SR 59,230, the corresponding Schild slope was significantly smaller than unity (Yamanishi *et al*., 2002a). The same investigators also reported a low potency of BRL 37,344 sensitive to SR 59,233 in the porcine bladder base (Yamanishi *et al*., 2002c). More recently, these authors also reported porcine bladder base relaxation by isoprenaline and salbutamol (Yamanishi *et al*., 2003a). CGP 20,712 did not inhibit the isoprenaline responses, whereas propranolol and ICI 118,551 caused inhibition, but with a Schild slope of less than unity; in contrast, ICI 118,551 inhibited the salbutamol responses with high potency and a Schild slope close to unity. Another group of investigators found an order of potency of isoprenaline = adrenaline ⩾ procaterol ⩾ BRL 37,344 > CGP 12,177 ⩾ salbutamol > CL 316,243 ⩾ noradrenaline; in this regard, BRL 37,344, CL 316,243 and, surprisingly, noradrenaline were reported to be partial agonists and CGP 12,177 was found to be a weak partial agonist (Badawi *et al*., 2005). Taken together, these findings suggest that both β_2-adrenoceptors and an additional subtype, possibly β_3-adrenoceptors, mediate porcine bladder relaxation.

Data from several other animal species are too limited or controversial to allow definitive conclusions. In guinea-pigs, a predominant role of β_1-adrenoceptors was proposed based upon relaxation by dobutamine, but not by BRL 37,344, salbutamol or clenbuterol, and antagonism of the isoprenaline, noradrenaline and adrenaline responses by atenolol (Yamamoto *et al*., 1998). Another study also proposed an involvement of β_1-adrenoceptors based upon partial antagonism of the isoprenaline response by metoprolol, but reported an even greater role of β_2-adrenoceptors based upon partial agonism by salbutamol and terbutaline and antagonism of the isoprenaline response by ICI 118,551 (Li *et al*., 1992). A more recent study based upon whole bladder contraction reported relaxation by noradrenaline and BRL 37,344, but not by formoterol (β_2-selective) (Gillespie, 2004). Limited data from one study in cats have suggested a predominant involvement of β_1-adrenoceptors (Nergardh *et al*., 1977). One study in ferrets has proposed a primary involvement of β_3-adrenoceptors based upon an agonist rank order of potency of BRL 37,344 > CGP 12,177 ⩾ isoprenaline ⩾ CL 316,243 > dobutamine ⩾ procaterol and upon antagonism of the isoprenaline response by SR 58,894, but not by CGP 20,712 or ICI 118,551 (Takeda *et al*., 2000a). One study in dogs reported an agonist rank order of potency of CL 316,243 > isoprenaline ⩾ CGP 12,177 > noradrenaline ⩾ dobutamine ⩾ procaterol ⩾ adrenaline, and that the isoprenaline-induced relaxation was inhibited with high potency by bupranolol, but not by CGP 20,712 or ICI 118,551 (Yamazaki *et al*., 1998); the same group later confirmed the rank order of CL 316,243 > dobutamine ≈ procaterol (Takeda *et al*., 2003), suggesting predominantly an involvement of β_3-adrenoceptors. A study in Cynomolgus monkeys found an agonist rank order of potency of isoprenaline > noradrenaline ⩾ CGP 12,177 > BRL 37,344 ⩾ adrenaline > dobutamine ⩾ salbutamol ⩾ procaterol, with the β_1-selective xamoterol being a very weak partial agonist; the effects of isoprenaline were inhibited by bupranolol, but not by CGP 20,712 or ICI 118,551 (Takeda *et al*., 2002a), suggesting a predominant involvement of a β_3-adrenoceptor.

Early reports on human bladder relaxation already proposed that this does not occur *via* a β_1- or β_2-adrenoceptor (Nergardh *et al*., 1977). Several more recent studies suggest that it indeed occurs *via* a β_3-adrenoceptor. Igawa *et al*. (1998) originally reported relaxation of the human bladder (inhibited by bupranolol), whereas dobutamine, procaterol and CGP 12,177 caused much smaller if any relaxation. Thereafter, they reported an agonist order of potency of BRL 37,344 ⩾ isoprenaline ⩾ noradrenaline ⩾ adrenaline ⩾ CGP 12,177 ⩾ CL 316,243; in that study, isoprenaline responses were inhibited by SR 58,894, but only poorly by ICI 118,551 and not at all by CGP 20,712 (Figure 2) (Igawa *et al*., 1999). Another study from the same group reported an order of BRL 37,344 ⩾ isoprenaline > CGP 12,177 ⩾ CL 316,243, with all but isoprenaline being partial agonists (Igawa *et al*., 2001). Another study reported a rank order of potency of BRL 37,344 > CGP 12,177 > isoprenaline, with the former two being partial agonists only, and the β_3-adrenoceptor agonist ZD 7114 being

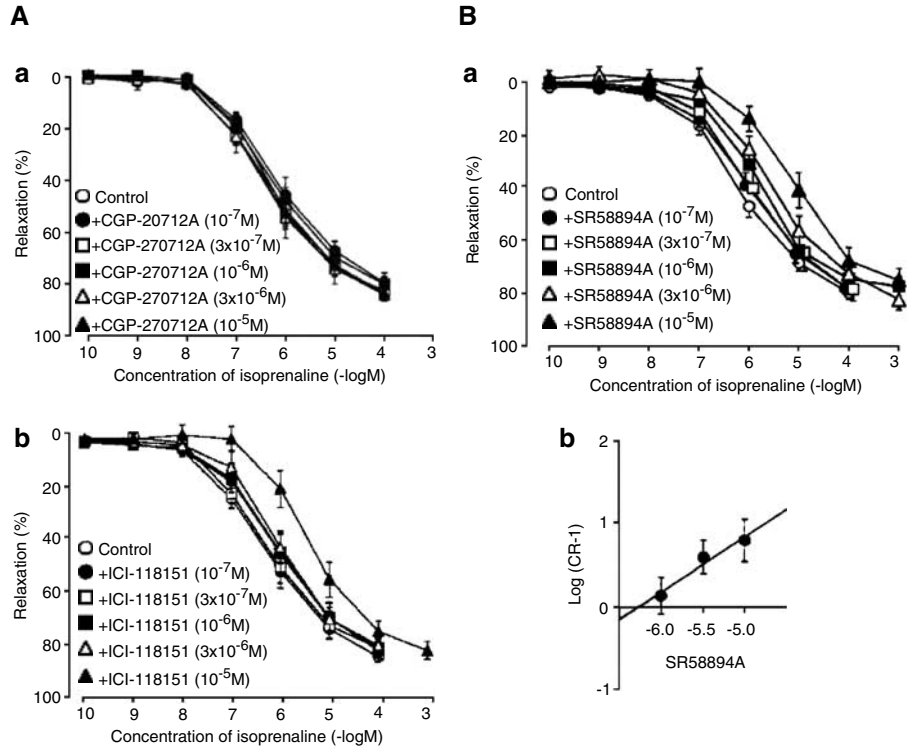

Figure 2 Inhibition of isoprenaline-induced relaxation of human bladder detrusor by the β_1-antagonist CGP 20,712, the β_2-antagonist ICI 118,551 and the nonselective antagonist SR 58,894. Taken with permission from Igawa *et al.* (1999).

a very poor partial agonist; the isoprenaline responses were inhibited by SR 59,230, but not by butoxamine and atenolol (Takeda *et al.*, 1999). In another study, isoprenaline and the β_3-adrenoceptor selective agonist L 755,507, but not dobutamine or clenbuterol, relaxed carbachol-contracted human bladder strips (Nomiya & Yamaguchi, 2003). A very recent study reported a rank order of potency of isoprenaline > procaterol = CL 316,243 = salbutamol, with the latter three compounds being considerably less effective than isoprenaline (Badawi *et al.*, 2005). Finally, GS 332 was found to be more potent in the human bladder than clenbuterol in another study (Morita *et al.*, 2000). In agreement with the predominant expression of β_3-adrenoceptor mRNA in the human bladder (see above), these data demonstrate that this subtype is also most important for bladder relaxation *in vitro*. With the possible exception of ferrets and monkeys, the role of this subtype in other animal species is less prominent.

In vivo *function* Functional *in vivo* effects on bladder function can be assessed in several ways. Noninvasive studies frequently look at micturition frequency, which is a key symptom of OAB (see Abrams *et al.*, 2002). Invasive studies are based upon the insertion of a catheter coupled to a pressure transducer into the bladder and subsequent filling of the bladder endogenously or by installation of fluid. This allows various types of measurements, including the frequency of bladder contractions, maximum detrusor pressure, filling volume at first contraction or bladder compliance, all of which are typically also assessed in urodynamic studies in humans (see Abrams *et al.*, 2002). Moreover, it should be considered that the effects of systemically administered drugs on bladder function are not necessarily mediated by drug targets located in the bladder (see the above section on bladder α_1-adrenoceptors). Finally, the use of anaesthetized vs conscious animals may differentially affect the endogenous sympathetic tone.

Studies in rats (Lecci *et al.*, 1998; Takeda *et al.*, 2000b; 2003; Kaidoh *et al.*, 2002; Tucci *et al.*, 2002), ferrets (Takeda *et al.*, 2000a) and monkeys (Takeda *et al.*, 2002a) demonstrate that β-adrenoceptor agonists such as isoprenaline can reduce intravesical pressure, indicating that this is a consistent feature in biology. On the other hand, propranolol had little, if any, effects on bladder function on its own (Durant *et al.*, 1988), indicating that either there is little endogenous β-adrenergic tone under the chosen experimental conditions and/or that the receptor mediating the bladder effects is propranolol-insensitive, that is, distinct from β_1- and β_2-adrenoceptors. Consistent with the latter possibility, neither i.v. terbutaline nor oral propranolol affected intra-vesical pressure in healthy women (Thind *et al.*, 1993b), but both drugs caused small increases in bladder volume in another study in healthy women (Norlen *et al.*, 1978).

A comparison of intra-peritoneal vs intra-thecal administration of isoprenaline in conscious, chronically instrumented rats with measurement of the contraction frequency of the urinary bladder demonstrated a peripheral site of action (Durant *et al.*, 1988). This is consistent with the finding that β-adrenoceptor agonists reduce bladder pressure both in conscious (Kaidoh *et al.*, 2002) and anaesthetized rats (Lecci *et al.*, 1998; Takeda *et al.*, 2000b; 2003; Tucci *et al.*, 2002).

In a model of distension-induced bladder activity under isovolumetric conditions in urethane-anaesthetized rats, the reduction of intra-vesical pressure by i.v. isoprenaline decreased with increasing intra-vesical volumes (Lecci *et al.*,

1998), possibly reflecting a physiological increase in endogenous β-adrenergic tone with increased bladder filling. In pentobarbital-anaesthetized rats, isoprenaline-induced reduction of bladder tone was attenuated by the cyclooxygenase inhibitor indomethacin, the Ca^{2+} flux blocker ruthenium red and the neurokinin A receptor antagonist MEN-10376, whereas the phosphodiesterase inhibitor papaverine did not affect them (Tucci *et al*., 2002). These data were interpreted to suggest that β-adrenoceptor-mediated bladder relaxations *in vivo* involve prostaglandins, neurokinin A and capsaicin-sensitive nerve fibres. Moreover, the lack of effect of papaverine is consistent with a cAMP-independent relaxation that has been demonstrated *in vitro* (Frazier *et al*., 2005a; Uchida *et al*., 2005).

In urethane-anaesthetized rats, isoprenaline, the β_2-agonist procaterol and the β_3-agonist CL 316,243 dose-dependently lowered intra-vesical pressure; CL 316,243 also increased bladder capacity and micturition intervals and reduced micturition pressure, whereas procaterol only increased bladder capacity and residual volume (Takeda *et al*., 2000b). Neither drug altered the total micturition volume, but their combination had somewhat greater effects on micturition interval, bladder capacity and residual volume than either drug alone. In conscious, unrestrained rats, i.v. procaterol did not affect voiding pressure relative to vehicle, and had little effect on bladder capacity, whereas CL 316,243 had no effect on bladder capacity but reduced voiding pressure (Kaidoh *et al*., 2002). Both procaterol and CL 316,243 reduced intra-vesical pressure in another study in urethane-anaesthetized rats; the procaterol effect was inhibited by ICI 118,551 and the CL 316,243 effect by the β_3-adrenoceptor antagonist L 748,337, whereas neither antagonist affected the response to the other agonist (Takeda *et al*., 2003). In pentobarbital-anaesthetized ferrets, isoprenaline and CL 316,243 dose-dependently reduced bladder pressure, whereas dobutamine and procaterol had little effect (Takeda *et al*., 2000a). Taken together, these data suggest that both β_2- and β_3-adrenoceptors contribute to bladder relaxation in rats *in vivo*, whereas only β_3-adrenoceptors are involved in ferrets. Both conclusions are consistent with the available *in vitro* data (see above).

In this context, it is interesting to note that the β_3-adrenoceptor agonists (in contrast to nonsubtype-selective or β_2-selective agonists) consistently had only small, if any, cardiovascular effects in the above studies (Takeda *et al*., 2000b; 2003; Kaidoh *et al*., 2002), indicating a possible safety advantage. On the other hand, two β_3-adrenoceptor agonists, ZD 7114 and ZD 2079, were reported to induce cystitis and renal tubular necrosis upon chronic dosing in male and female rats (Waghe *et al*., 1999), but it remains unclear whether this is a specific effect of these two compounds or related to their mechanism of action; moreover, it is unclear whether this is limited to rats or can be extrapolated to other species.

Regulation of receptor expression and function A possible gender effect on β-adrenoceptor-mediated regulation of bladder function has been studied in rabbits and rats. Radioligand-binding studies with [^{3}H]dihydroalprenolol as the radioligand have found a significantly greater receptor number in young female as compared to young male rabbits in the bladder base, but no such differences were seen in the bladder base of older rabbits or in the bladder dome of either age group (Latifpour *et al*., 1990). Another study with the same radioligand confirmed a greater receptor number in the trigonal part (but not the detrusor) of young adult female as compared to male rabbits (Morita *et al*., 1998). Within the same study, this was confirmed functionally by a greater isoprenaline-induced relaxation in female than in male trigonal, but not detrusor muscle. Similarly, a study on ovariectomized Wistar rats reported a reduced potency for BRL 37,344 in relaxing bladder strips as compared to control- or oestrogen-treated ovariectomized rats; similar differences for isoprenaline did not reach statistical significance (Matsubara *et al*., 2002). On the other hand, relaxant responses for the weak partial agonist CGP 12,177 were reduced in female relative to male Wistar rats, but no such alterations was seen for the agonists BRL 37,344, isoprenaline and noradrenaline (Frazier *et al*., 2005b).

A regulation of β-adrenoceptor responsiveness with age has been demonstrated in several species. A binding study with [^{3}H]dihydroalprenolol reported that the number of β-adrenoceptors increased in rabbit bladder dome and base with age (Latifpour *et al*., 1990). In contrast, a study using [^{125}I]iodopindolol in 1- vs 3- vs 22-month-old male Fischer 344 rats reported an age-related decrease in receptor density (Nishimoto *et al*., 1995). A similar decrease in [^{3}H]dihydroalprenolol-binding sites was also reported for human bladder (Li *et al*., 2003). Consistent with these findings, an age-related reduction of isoprenaline-stimulated cAMP formation has been found in a comparison of bladders from 3-, 6- and 24-month-old male Fischer 344 rats (Derweesh *et al*., 2000). The latter was accompanied by an increase in the expression of α-subunits of G_s, G_o and G_i proteins, with the latter two increasing more than G_s and hence shifting the overall balance towards inhibition rather than stimulation of adenylyl cyclase. In line with these biochemical findings, it was reported from a comparison of 1- vs 3- vs 22-month-old male Fischer 344 rats that bladder relaxation by noradrenaline or isoprenaline against KCl-induced tone was attenuated, involving a reduction in agonist potency and maximum effects; isoprenaline effects against field stimulation-induced tone were similarly reduced (Nishimoto *et al*., 1995). Within that study, relaxation responses to forskolin, but not those to dibutyryl-cAMP, were also reduced with age, indicating that an alteration prior to cAMP formation rather than in cAMP responsiveness is involved. While these biochemical and functional studies in male Fischer 344 rats are consistent with a reduced β-adrenoceptor function with age, not all studies have confirmed that. Thus, one study in 7- vs 17- vs 29-month-old male Fischer 344 rats detected no alteration in the potency or efficacy of isoprenaline to relax isolated bladder strips (Kolta *et al*., 1984). Similarly, a study in 3- vs 23-month-old male Wistar rats reported similar concentration-dependent bladder strip relaxation by isoprenaline, noradrenaline, BRL 37,344 and CGP 12,177 in both age groups (Frazier *et al*., 2005b). A study in 10- vs 30-month-old female Wistar/Rij rats also found similar isoprenaline-induced bladder strip relaxation in both age groups (Lluel *et al*., 2000). Finally, a study comparing newborn, 1- and 4-month-old Sprague–Dawley rats also did not detect alterations of β-adrenoceptor-mediated bladder relaxation (Tugay *et al*., 2003), a finding probably more related to development than to ageing. In this context, it should be noted that studies on age-related differences of muscarinic receptor responsiveness in the bladder have found major strain differences, with Wistar rats most closely

resembling the situation in humans (Schneider *et al*., 2005). A single and limited study in humans has reported that bladder relaxation responses to isoprenaline and BRL 37,344 and also receptor-independently to forskolin and dibutyryl-cAMP are lower in a group of subjects in their mid-60s than in those in their late 20s (Li *et al*., 2003), indicating that the observed difference may at least partly relate to an overall reduced ability to relax rather than a specific β-adrenoceptor desensitization.

Several studies have investigated the effects of β-adrenoceptor agonists in animal models of bladder dysfunction. Some of them compared such effects with those in healthy animals to test the possible alterations by disease, whereas other studies looked at the pathological condition only to determine whether β-adrenoceptor agonists might be effective therapeutics in such settings. Spontaneously hypertensive rats are a genetic animal model, which exhibits several features of OAB, including increased urinary frequency and reduced bladder capacity. A comparison of male spontaneously hypertensive with normotensive Wistar Kyoto rats detected a reduced bladder relaxation in response to noradrenaline and isoprenaline, but not to the partial agonist BRL 37,344 (Frazier *et al*., 2005b). OAB-like symptoms can also occur secondarily to bladder outlet obstruction. CL 316,243 dose-dependently inhibited spontaneous bladder contraction in obstructed rats, but a direct comparison with healthy rats (who have much less if any such spontaneous contractions) was not reported (Woods *et al*., 2001). When bladder hyper-reflexia was induced by intra-vesical installation of acetic acid, CL 316,243 also concentration-dependently reduced bladder contractions; comparison to the obstruction data from the same study indicates that the hyper-reflexic model may be more sensitive to this agonist (Woods *et al*., 2001). Bladder hyperactivity can also be induced by intra-vesical installation of prostaglandin E_2. In this model, CL 316,243 dose-dependently increased micturition interval and micturition volume and decreased basal pressure, whereas threshold pressure and micturition pressure were not affected; on the other hand, procaterol reduced threshold pressure, but did not significantly affect the other parameters (Takeda *et al*., 2002b). Bladder hyper-reflexia can also be induced by cerebral infarction, which impairs some of the central nervous control of the bladder. While CL 316,243 had little effect on bladder capacity in control animals, it dose-dependently restored the reduced bladder capacity in cerebral infarction rats, and a similar restoration of bladder capacity was seen with procaterol; neither drug normalized voiding pressures within the tested dose range (Kaidoh *et al*., 2002).

Few studies have looked into alterations of β-adrenoceptor responsiveness in the bladder of patients. A limiting factor of all these studies is the problem of obtaining tissue from matched healthy controls. Apparently tumour-free tissue from cancer patients is most frequently used as control; while this appears the only feasible option, it remains unclear how representative such tissue is for healthy subjects. One study compared the relaxation of isolated bladder strips without precontraction by the β-adrenoceptor agonists isoprenaline, BRL 37,344, CL 316,243 and CGP 12,177 in patients with low bladder compliance, hyperreflexic bladders and controls; agonist potency was similar in all three groups for each agonist, and maximum effects were also similar across groups for the agonists, except for an increased effect of CGP 12,177 in low-compliance bladders (Igawa *et al*., 2001). Another study has reported on the relaxation of field stimulation-contracted bladder strips from patients with urodynamically confirmed urge incontinence with those from continent patients without a history of incontinence; clenbuterol caused only weak relaxation in control subjects at both 1 and 40 Hz stimulation, but significantly greater relaxation in strips from incontinent patients (Hudman *et al*., 2001). A comparison of bladder from males with and without bladder outlet obstruction detected statistically significant differences in the abundance of β_1-, β_2- or β_3-adrenoceptor mRNA between groups; similarly, potency and maximum effects of isoprenaline and the β_3-selective L 755,507 were similar in both groups (Nomiya & Yamaguchi, 2003). Taken together, the limited available animal and human data do not provide conclusive evidence for an alteration of β-adrenoceptor function in states of bladder dysfunction.

Clinical implications The available data demonstrate that β-adrenoceptor agonists relax urinary bladder from various species including humans. In humans, this occurs largely, if not exclusively, *via* a β_3-adrenoceptor. Several animal studies suggest that selective β_3-agonists will have much fewer, if any, cardiovascular side effects as compared to agonists acting on other β-adrenoceptor subtypes (Takeda *et al*., 2000b; 2003; Kaidoh *et al*., 2002). Moreover, β-adrenoceptor agonists improved symptoms in various rat models of bladder dysfunction, and small pilot studies reported beneficial effects with terbutaline (Lindholm & Lose, 1986) and clenbuterol (Gruneberger, 1984). Against this background, several pharmaceutical companies are developing β_3-adrenoceptor agonists for the treatment of OAB. Some of them have recently reported positive proof-of-concept studies with their selective agonists in OAB patients; while none of the underlying studies have published in the peer-reviewed literature, such findings would seem to indicate that bladder β_3-adrenoceptors are a potentially important drug target.

Urethra

The urethra functions not only as a passive conduit for the urine being passed from the bladder but also actively contributes to bladder outlet resistance and hence the maintenance of continence during the filling/storage phase of the micturition cycle. In contrast to the other tissues covered in this manuscript, the urethra contains both smooth muscle, frequently referred to as the 'internal urethral sphincter', and striated muscle, frequently referred to as the 'external urethral sphincter'. Since sympathetic fibres primarily innervate the smooth muscle, much of the data reviewed below refer to the smooth muscle portion of the urethra only. However, it should be noted that striated urethral muscle also expresses adrenoceptors and that centrally located adrenoceptors may indirectly affect striated muscle function in the urethra by modulating the activity of the somatic pelvic nerves (see Michel *et al*., 2005c). Moreover, studies on the urethra have often been used as a substitute for the prostate in the evaluation of α_1-adrenoceptor antagonist for the treatment of LUT symptoms suggestive of BPH.

α_1-Adrenoceptors

mRNA and protein expression The presence of α_1-adrenoceptors in the urethra has been assessed at the mRNA and protein level. Studies in rats have detected a rank order of abundance of $\alpha_{1A} > \alpha_{1B} \geqslant \alpha_{1D}$ using real-time PCR (Yono *et al*., 2004). In the human proximal urethra, RNase protection assays detected the α_{1A}-adrenoceptor as the most abundant subtype in male and female samples, whereas α_{1D}-adrenoceptor mRNA was seen only in female samples and α_{1B}-adrenoceptor mRNA in neither; this resulted in an $\alpha_{1A} : \alpha_{1B} : \alpha_{1D}$ ratio of 100 : 0 : 0 in males and 90 : 0 : 10 in females (Nasu *et al*., 1998). *In situ* hybridization studies confirmed these findings and localized the α_{1A}-signal to the urethral smooth muscle (Nasu *et al*., 1998). *In situ* hybridization studies in the penile urethra of the rhesus monkey have found no α_{1A}-adrenoceptor signal in the urothelium or surrounding connective tissue, but in the smooth muscle and longitudinal striated muscle layers (Walden *et al*., 1997), with the functional significance of the latter remaining unclear.

Receptor autoradiography studies under conditions preferentially detecting α_{1A}-adrenoceptors confirmed the presence of this subtype at the protein level in smooth and longitudinal muscle layers of the monkey urethra (Figure 1) (Walden *et al*., 1997). Radioligand-binding studies in tissue homogenates confirmed the presence of α_1-adrenoceptors in the rabbit urethra; these studies demonstrated an abundance similar to that in bladder base and exceeding that in the detrusor (Andersson *et al*., 1984; Larsson *et al*., 1986; Latifpour *et al*., 1990; Testa *et al*., 1993). Within the rabbit urethra, proximal and distal parts were reported to express similar α_1-adrenoceptor densities (Larsson *et al*., 1986). A study comparing urethral α_1-adrenoceptors in multiple species in radioligand-binding experiments found a rank order of abundance of rat > human > dog > rabbit; based upon a lack of their inactivation by chloroethylclonidine, it was proposed that the α_1-adrenoceptors in these species belong largely, if not exclusively, to the α_{1A}-subtype (Testa *et al*., 1993). Thus, the urethra of various species including humans contains α_1-adrenoceptors at the mRNA and protein levels, which appear to largely belong to the α_{1A}-subtype.

In vitro *and* in vivo *functions* Various studies have assessed the role of α_1-adrenoceptors in the contractile tone of the urethra. Most of these were done in rabbits, but limited data have also been presented for rats, dogs and humans. In contrast to the bladder and prostate (see above and below), the adrenoceptor-mediated contraction of the rabbit urethra involves both α_1- and α_2-adrenoceptors (Ueda *et al*., 1984; Larsson *et al*., 1986). Field stimulation studies indicate that the proximal urethra appears largely under the control of the parasympathetic nervous system, whereas the medial and distal urethra are to a larger extent controlled by the sympathetic nervous system (Deplanne *et al*., 1998). The overall contribution of an α-adrenoceptor mechanism relative to muscarinic mechanism to nerve stimulation-induced urethral contraction appears limited (van der Werf & Creed, 2002). Nevertheless, it was found that the potency of an α_1-adrenoceptor agonist did not differ between rabbit proximal and distal urethra (Larsson *et al*., 1986). A study involving multiple subtype-selective antagonists found that the contraction of the rabbit urethra is mediated by the α_{1A}-adrenoceptor (Testa *et al*., 1993). This was confirmed by a high affinity of the moderately α_{1A}-selective tamsulosin in other studies on rabbit urethra (Honda & Nakagawa, 1986). However, multiple studies have demonstrated that the α_{1A}-adrenoceptor mediating the contraction of rabbit urethra has rather low affinity for prazosin (Lefevre-Borg *et al*., 1993; Testa *et al*., 1993; Deplanne & Galzin, 1996; van der Graaf *et al*., 1997), demonstrating that it exhibits the α_{1L}-phenotype of the α_{1A}-adrenoceptor (see also sections on bladder and prostate). Mechanistically, it was reported that α_{1A}-adrenoceptor-mediated contraction of the rabbit urethra is moderately sensitive to inhibition by the Ca^{2+}-entry blocker nifedipine (Testa *et al*., 1993). Apparently, α_1-adrenoceptor-mediated urethral contraction has also been demonstrated in rats (Lluel *et al*., 2003a), dogs (Testa *et al*., 1993) and humans (Kunisawa *et al*., 1985). However, the latter was reported to differ from the situation in rabbits in two important ways: Firstly, contraction of the human urethra appeared to involve only α_1- and no α_2-adrenoceptors. Secondly, the α_1-adrenoceptor-mediating contraction of the human urethra exhibited high affinity for prazosin, indicating that it does not represent the α_{1L}-phenotype repeatedly found in rabbits.

Two studies in anaesthetized cats found that hypogastric nerve-induced urethral contraction is α_1-adrenoceptor-mediated (Lefevre-Borg *et al*., 1993; Springer *et al*., 1994). The α_1-adrenoceptor agonist midodrine also increased urethral pressure in anaesthetized rats (Takeda *et al*., 2003). Studies with several subtype-selective α_1-adrenoceptor and 5-HT receptor antagonists demonstrate that $\alpha_{1A/D}$-adrenoceptors and 5-HT_{1A} receptors are involved in urethral contraction *via* the micturition reflex; the anatomical location of these receptors has not been fully clarified (Conley *et al*., 2001). Studies in healthy women found that prazosin ($1\,mg\,kg^{-1}$) reduced the intra-urethral static pressure, but did not affect responses to coughing or squeezing (Thind *et al*., 1992; 1993a). Numerous other *in vivo* studies on the effects of α_1-adrenoceptor agonist and antagonists on intra-urethral pressure (IUP) have been performed in animals, but the observed effects can largely be attributed to effects on prostatic rather than urethral α_1-adrenoceptors (Akiyama *et al*., 1999), and hence will be discussed in the prostate section of this manuscript (see below).

Regulation of receptor expression and function Few studies have investigated a physiological or pathophysiological regulation of urethral α_1-adrenoceptors. They were reported not to be affected by gender at the mRNA level in humans (Nasu *et al*., 1998) or at the protein level in rabbits (Latifpour *et al*., 1990). In contrast, α_1-adrenoceptor-mediated urethral contraction was reported to be markedly reduced in 24- vs 6-month-old rats (Lluel *et al*., 2003a). One study reported that chronic treatment with a high dose of the antagonist doxazosin upregulates α_{1A}-adrenoceptor mRNA in rat urethra; however, this finding remains difficult to interpret, since several other dosing regimens within the same study did not reveal such upregulation (Yono *et al*., 2004).

Clinical implications Based upon the contribution of the urethra to bladder outlet resistance and the role of α_1-adrenoceptors in controlling urethral tone, α_1-adrenoceptors are a potential target in the treatment of stress incontinence. Several α_1-adrenoceptor agonists, particularly those

with selectivity for the α_{1A}-subtype, have been developed preclinically for this indication, including NS-49 (Obika *et al*., 1995), A-61603 (Knepper *et al*., 1995) and Ro 115–1240 (Blue *et al*., 2004). The latter compound has been successfully tested in a clinical proof-of-concept study in stress incontinence patients (Musselman *et al*., 2004), but its clinical development has been discontinued due to undisclosed reasons. Whether the beneficial effects of the combined serotonin/noradrenaline uptake inhibitor duloxetine in stress incontinence apart from its spinal cord-mediated enhancement of the somatic pudendal nerve activity also involve a direct effect on the urethral smooth muscle remains to be determined (see Michel *et al*., 2005c).

α_2-*Adrenoceptors*

mRNA and protein expression To the best of our knowledge, the presence of α_2-adrenoceptor subtype mRNA in the urethra has not been reported. At the protein level, urethral α_2-adrenoceptors have repeatedly been identified in radioligand-binding studies in rabbits (Andersson *et al*., 1984; Larsson *et al*., 1986; Latifpour *et al*., 1990). One of these studies found that the density of α_2-adrenoceptors increases from the proximal to the distal urethra (Larsson *et al*., 1986). Based upon prazosin affinity, the urethral α_2-adrenoceptors appear to belong largely, if not exclusively, to the α_{2A}-subtype (Latifpour *et al*., 1990), which is consistent with those in bladder and prostate (see above and below). The density of rabbit urethral α_2-adrenoceptors appears not to be regulated by gender or age (Latifpour *et al*., 1990).

In vitro *and* in vivo *function* Functionally, in contrast to the studies in the bladder and prostate (see above and below), α_2-adrenoceptors can elicit urethral contraction, which is quantitatively at least as strong as that mediated by α_1-adrenoceptors, in rabbits (Andersson *et al*., 1984; Ueda *et al*., 1984; Larsson *et al*., 1986) and horses (Garcia-Sacristan *et al*., 1984). In line with the finding that α_2-adrenoceptor expression increases in the distal urethra, an enhanced α_2-adrenoceptor-mediated contraction was found in this part of the rabbit urethra (Larsson *et al*., 1986). In line with the *in vitro* data in rabbits, *in vivo* studies in dogs reported that i.v. clonidine can increase IUP *via* an α_2-adrenoceptor, and that this effect accounted for about 50% of that of adrenaline; whether the difference between the two is due to an α_1-adrenoceptor component in the adrenaline response or due to the partial agonism of clonidine remains to be determined (Shapiro *et al*., 1987). In contrast to rabbits and dogs, however, no α_2-adrenoceptor-mediated contraction of the human urethra was found *in vitro* (Kunisawa *et al*., 1985). Therefore, little consideration has been given to the possibility of urethral α_2-adrenoceptors being a potential therapeutic target in urological disease.

β-*Adrenoceptors*

mRNA and protein expression To the best of our knowledge, the presence of β-adrenoceptor subtype mRNA in the urethra has not been reported. We are aware of only three studies that have quantified urethral β-adrenoceptors at the protein level using radioligand binding. One study reported a similar receptor density in the urethra of young and old male and female rabbits (Latifpour *et al*., 1990). Within the study, the β-adrenoceptor density in the urethra was somewhat lower than that in the bladder base and much lower than in the detrusor. In all four groups, the β_2-selective antagonist ICI 118,551 had a much higher affinity in competition-binding studies than the β_1-selective antagonist ICI 89,406, indicating that the urethral β-adrenoceptors in the rabbit urethra belong largely, if not exclusively, to the β_2-subtype. A study in female pigs demonstrated binding sites with a low affinity for the β_1-selective CGP 20,712A, whereas the β_2-selective ICI 118,551 yielded biphasic fits with approximately 30% high-affinity sites; SR 59,230A, an antagonist that is often used to block β_3-adrenoceptors but is not selective for this subtype and actually has slightly higher affinity for β_1- and β_2-adrenoceptors (Hoffmann *et al*., 2004), yielded monophasic competition curves (Yamanishi *et al*., 2002c). These data are difficult to interpret (see the above discussion of data on bladder base from same paper). The low affinity of CGP 20,712A appears to exclude the presence of β_1-adrenoceptors. While it would be logical to assume that the low-affinity component of the ICI 118,551 competition curves represents β_3-adrenoceptors, the study has used [^{3}H]dihydroalprenolol as the radioligand, which is unlikely to detect β_3-adrenoceptors (Hoffmann *et al*., 2004). Finally, one study has used [^{125}I]iodocyanopindolol to detect β-adrenoceptors in the human external urethral sphincter, but no detailed subtype characterization was performed (Morita *et al*., 2000). Therefore, the identity of the β-adrenoceptor subtype in the urethra remains unclear.

In vitro *function* Functional *in vitro* studies on urethral β-adrenoceptors have been performed in rat, dog, pig and horse. In rats and dogs, the maximum relaxant effects of several β-adrenoceptor agonists were only about half of those seen in bladder detrusor or trigone within the same study; the maximum relaxant effects were greater in the proximal than in the distal canine urethra (Takeda *et al*., 2003). Attempts to identify the β-adrenoceptor subtype mediating urethral relaxation have been based upon rank orders of potency of subtype-selective agonists and on their inhibition by subtype-selective antagonists. In the rat urethra, the β_2-selective agonist procaterol was more potent than the β_3-selective CL 316,243, and the β_1-selective dobutamine was least potent (Takeda *et al*., 2003). Limited data in the equine urethra indicate involvement of a β_2-adrenoceptor (Garcia-Sacristan *et al*., 1984). In the canine urethra, the agonist rank order of potency was procaterol > dobutamine = CL 316,243, which clearly differed from that observed in the detrusor within the same study, where CL 316,243 had been the most potent agonist (Takeda *et al*., 2003). In the porcine urethra, the β_3-selective BRL 37,344 was more potent than the nonselective isoprenaline, with the β_2-selective salbutamol being least potent (Yamanishi *et al*., 2003b). BRL 37,344 relaxed the urethra about 300 times more potently than the bladder base (Yamanishi *et al*., 2002c). The nonselective antagonist propranolol inhibited isoprenaline-induced relaxation with high potency (pA_2 8.55), but had a Schild slope of less than unity (Yamanishi *et al*., 2003b). While even high concentrations of the β_1-selective CGP 20,712A had no effect against isoprenaline, the β_2-selective ICI 118,551 inhibited the effects of both salbutamol and isoprenaline with high potency and a Schild slope close to unity (Yamanishi *et al*., 2003b). SR 59,230A inhibited the effects of BRL 37,344 (Yamanishi *et al*.,

2002c) and isoprenaline (Yamanishi *et al*., 2003b), with apparent pA_2 values of 7.72 and 7.38, respectively, but in either case the Schild slope was less than unity, which makes interpretation of the data difficult. Taken together, these data demonstrate that the functional importance of β-adrenoceptors for smooth muscle tone in the urethra, particularly in its distal parts, is less than in the bladder. This is consistent with the lower β-adrenoceptor expression level in the urethra as compared to the bladder (Latifpour *et al*., 1990). This is also in line with studies demonstrating that the nerve stimulation-induced relaxation of the urethra is largely mediated by NO, whereas β-adrenergic mechanisms account for a minor component only (van der Werf & Creed, 2002). Relaxation of the rat, dog and pig urethra appears to involve a strong β_2-adrenoceptor component; an additional involvement of a β_3-adrenoceptor appears possible, particularly in the porcine urethra, whereas β_1-adrenoceptors do not appear important in either species. Since the urethra also contains striated muscle (external urethral sphincter), it appears interesting to note that β-adrenoceptor stimulation has been reported to cause contraction of the striated urethral muscle (possibly *via* β_2-adrenoceptors), which may contribute to the overall effects on urethral resistance (Morita *et al*., 2000).

In vivo *function* A possible *in vivo* role of urethral β-adrenoceptors has been investigated in rats, cats and humans. In anaesthetized rats the β_2-agonist procaterol reduced urethral pressure, while the β_3-agonist CL 316,243 had less, if any, effects; the pressure reductions by procaterol were inhibited by the β_2-antagonist ICI 118,551 (Takeda *et al*., 2003). Hypogastric nerve stimulation in cats caused propranolol-sensitive urethral relaxation, which was further enhanced in the presence of the noradrenaline uptake inhibitors nisoxetine and tomoxetine (Springer *et al*., 1994). In healthy women, systemic administration of the β_2-adrenoceptor agonist terbutaline reduced resting urethral pressure, whereas the antagonist propranolol had no effect (Thind *et al*., 1993a, b).

Clinical implications In conclusion, the stimulation of β-adrenoceptors by exogenous agonists can induce urethral relaxation, an effect predominantly involving the β_2-subtype. Endogenous catecholamines do not appear to have major effects on urethral β-adrenoceptors. Since even the effects of exogenous agonists on the urethra are small relative to those on the bladder, it appears unlikely that the use of β-adrenoceptor agonists, particularly those selective for the β_3-subtype, for the treatment of OAB will have no major adverse effects on urethral tone.

Prostate

In contrast to bladder and urethra, which are largely muscular tissues, the prostate contains both stromal, that is, smooth muscle, and epithelial, that is, glandular, elements, which serve different functions. The ratio of smooth muscle and glandular elements differs markedly between species, and few species mimic the strong stromal component in the human prostate (Lepor *et al*., 1994). The histological state of human BPH is associated with a relative increase in the smooth muscle component of the prostate (Bartsch *et al*., 1979; Shapiro *et al*., 1992). The enlarged prostate is believed to contribute to increased bladder outlet resistance in two ways. Firstly, the enlargement itself can narrow the urethral lumen, and this is referred to as the static component of bladder outlet obstruction. Secondly, the contraction of bladder smooth muscle can additionally narrow the urethral lumen, and this is referred to as the dynamic component of bladder outlet obstruction. The high prevalence of BPH in elderly men has sparked a large number of studies into prostatic adrenoceptors, particularly α_1-adrenoceptors.

α_1-Adrenoceptors

mRNA expression Prostatic α_1-adrenoceptors have received considerable attention in the last decade due to the clinical success of α_1-adrenoceptor antagonists in the treatment of symptoms of BPH (see Roehrborn & Schwinn, 2004). The presence of α_1-adrenoceptor subtype mRNA has been assessed in the prostate of various species, including rats, rabbits, monkeys and humans. An early study on rat prostate based upon a competitive RT–PCR technique described the presence of α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor mRNA in a 99 : 1 : 0% ratio (Scofield *et al*., 1995). Other studies in rat prostate also using RT–PCR (Rokosh *et al*., 1994; Homma *et al*., 2000) or real-time PCR (Foster *et al*., 2004) did not confirm this strong dominance and rather described a roughly similar abundance of all three subtypes. On the other hand, an *in situ* hybridization study on rat prostate detected only the α_{1A}-adrenoceptor, which was primarily localized in the prostatic smooth muscle cells (Walden *et al*., 1997). The same study reported similar findings for the monkey prostate (Walden *et al*., 1997). A study in rabbits has looked only at the α_{1D}-subtype mRNA, and found this to be highly abundant in the prostate, that is, at a relative density only surpassed by the vas deferens and thoracic aorta in a panel of 16 tissues (Suzuki *et al*., 1997).

Owing to the obvious therapeutic implications, various studies have reported on α_1-adrenoceptor subtype mRNA in the human prostate. While early studies based upon RT–PCR have detected only α_{1A}-adrenoceptor mRNA in the human prostate (Hirasawa *et al*., 1993), later studies using Northern blots, RT–PCR or RNase protection assays have typically also detected α_{1D}-adrenoceptor mRNA (Price *et al*., 1993; Faure *et al*., 1994; Weinberg *et al*., 1994; Tseng-Crank *et al*., 1995; Moriyama *et al*., 1996; Nasu *et al*., 1996). The overall data indicate that the approximate relative ratio of α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor mRNA in the human prostate is 70 : 0 : 30%. In line with this relative dominance of the α_{1A}-adrenoceptor at the mRNA level, an *in situ* hybridization study has detected only this subtype (Walden *et al*., 1997). The α_1-adrenoceptor subtype expression appears similar in the peri-urethral, base, apex and lateral lobe regions of the human prostate (Faure *et al*., 1994) or in its urethral, central and peripheral regions (Moriyama *et al*., 1998). At the cellular level, α_{1A}-adrenoceptor mRNA expression was found both in stromal (smooth muscle) and epithelial (glandular) cells (Tseng-Crank *et al*., 1995). Messenger RNA for α_{1B}- and α_{1D}-adrenoceptors was found in primary cultures of human prostatic smooth muscle cells in some (Boesch *et al*., 1999), but not other, studies (Tseng-Crank *et al*., 1995). Of note, at least four splice variants exist of the α_{1A}-adrenoceptor, and the human prostate expresses all of them, with variant 4 exhibiting the greatest abundance (Chang

et al., 1998). The epithelial prostate cancer cell lines PC3 and Du145 have been reported to express mRNA for all three α_1-adrenoceptor subtypes (Tseng-Crank *et al*., 1995). Taken together, at least in rats and humans considerable amounts of data demonstrate that the α_{1A}-subtype is the most abundant α_1-adrenoceptor at the mRNA level.

Protein expression The presence of α_1-adrenoceptors at the protein level has been investigated in radioligand-binding and receptor autoradiography studies in a variety of species, including rats, rabbits, pigs, dogs, monkeys and humans. While indirect comparisons between species are difficult, some studies have concomitantly investigated multiple species. Despite the major histological differences between species with regard to stromal content, it was reported that the density of α_1-adrenoceptor-binding sites differed only modestly among them, with a rank order of abundance of human$\geqslant$rat$\geqslant$dog$\geqslant$rabbit (Testa *et al*., 1993). Studies in human tissues have investigated the intra-prostatic distribution of α_1-adrenoceptors at the macroscopic and microscopic levels. One study reported that the α_1-adrenoceptor density was greater in the adenoma as compared to the submucosal tissue of the prostatic urethra in enucleated hyperplastic prostate tissue (Kawabe *et al*., 1990). On the other hand, similar receptor densities were found in the central and peripheral zones of BPH tissue (Chapple *et al*., 1989). Another study divided the prostate into eight different regions and detected similar α_1-adrenoceptor densities and similar phenylephrine-induced contractions in all of them (Lepor *et al*., 1993a). At the microscopic levels, receptor autoradiography studies have localized rat and human prostatic α_1-adrenoceptors predominantly to the stroma rather than the glandular tissue, and within the stroma largely to smooth muscle rather than fibroblasts or connective tissue (Chapple *et al*., 1989; Killam *et al*., 1995). This was confirmed at higher resolution using fluorescent approaches (MacKenzie *et al*., 2000).

The relative presence of α_1-adrenoceptor subtypes has been investigated in the prostate of various species. Early studies have used the ability of chloroethylclonidine to subtype-selectively alkylate α_1-adrenoceptors so as to identify subtypes in the prostate. However, this approach has limited validity because chloroethylclonidine can potentially alkylate all subtypes of α_1- and even α_2-adrenoceptors if incubation concentration, time and temperatures are high, long and warm enough, respectively (Michel *et al*., 1993). One study using an incubation of prostatic membranes with 10 μM chloroethylclonidine for 30 min at 37°C has reported that $>80\%$ of receptors in the rat and human, $>90\%$ of all receptors in the rabbit and almost all receptors in the canine prostate are chloroethylclonidine-insensitive, and proposed them to belong to the α_{1A}-subtype (Testa *et al*., 1993). On the other hand, a study using less stringent incubation conditions (10 μM chloroethylclonidine for 30 min at room temperature) reported only 77, 44 and 56% chloroethylclonidine-insensitive α_1-adrenoceptor-binding sites in rat, canine and human prostate, respectively (Lepor *et al*., 1994). Expectedly, studies using a tritiated form of moderately α_{1A}-selective tamsulosin as the radioligand have detected only chloroethylclonidine (10 μM for 10 min at 37°C) resistant sites in the rat prostate (Yazawa & Honda, 1993).

Later studies have rather used competition-binding experiments with subtype-selective drugs to characterize α_1-adrenoceptors in the prostate. One study in rats detected low affinity of the α_{1D}-selective BMY 7378 in rat prostate, indicating that this subtype is largely absent in rat prostate at the protein level; the same study detected monophasic competition curves of high affinity for the somewhat α_{1A}-selective 5-methylurapidil, suggesting that this is the most abundant subtype at the protein level in rat prostate (Deng *et al*., 1996). Supporting this conclusion, studies with [^{3}H]tamsulosin as the radioligand also found high affinity of the α_{1A}-selective WB 4101 and 5-methylurapidil (Yazawa & Honda, 1993). Studies in rabbit prostate also reported at least 50% of α_1-adrenoceptor-binding sites to have high affinity for WB 4101 and 5-methylurapidil (Hiraoka *et al*., 1995). One study in dog prostate detected mainly low-affinity sites for WB 4101 (Ohmura *et al*., 1993), but the poor selectivity of this drug makes the data difficult to interpret. Based upon competition by a single concentration of the highly α_{1A}-selective antagonist SNAP 5272, autoradiographic studies reported that this subtype accounts for at least 80% of α_1-adrenoceptor protein in the monkey prostate (Figure 1) (Walden *et al*., 1997). In an early study of the human prostate, the potency of a panel of six drugs (prazosin, phentolamine, 5-methylurapidil, urapidil, spiperone, WB 4101) to compete for [^{3}H]prazosin binding correlated best with that for binding to rabbit liver (α_{1A}-adrenoceptors), somewhat less with that for binding to chloroethylclonidine-treated rat hippocampus (α_{1D}-adrenoceptors) and worst with that for binding to rat liver (α_{1B}-adrenoceptors), suggesting that the receptors in the human prostate largely belong to the α_{1A}-subtype (Testa *et al*., 1993). A similar approach was used by other investigators, who substituted the prototypical animal tissues with cell lines transfected with the cloned human receptors; in such experiments, the affinity of a panel of 12 drugs, some of which, such as the stereoisomers of niguldipine, have much greater subtype selectivity than those used in earlier studies, correlated very well with that at α_{1A}-adrenoceptors, but not with that at either other subtype (Tseng-Crank *et al*., 1995). A study from our own laboratory has used narrowly spaced concentration increments of 5-methylurapidil, phentolamine, tamsulosin and WB 4101 in competition-binding studies with human prostate membranes (Michel *et al*., 1996). This has allowed detecting biphasic competition curves for all four agents despite their limited selectivity for α_{1A}-adrenoceptors, and the percentage of high-affinity sites for these agents ranged between 61 and 79%, confirming that the α_{1A}-adrenoceptor is the most abundant subtype in the human prostate (Figure 3). In conclusion, it appears that the α_{1A}-subtype is the most abundant α_1-adrenoceptor subtype in the rat and human prostate at the protein level, and limited data suggest a similar situation in rabbits and monkeys. The remaining receptors appear to belong largely to the α_{1B}-subtype, with α_{1D}-adrenoceptors apparently being absent at the protein level. While the large abundance of α_{1A}-adrenoceptor protein is in agreement with the mRNA data, no such agreement between mRNA and protein data exists for α_{1B}- and α_{1D}-adrenoceptors. The reasons for the poor detection of α_{1D}-adrenoceptor protein despite the presence of corresponding mRNA remain to be elucidated, but similar discrepancies for mRNA and protein expression of this subtype have been found in many other tissues of several species (Yang *et al*., 1997; 1998b).

In vitro *function* Studies in guinea-pig prostate have demonstrated that α_1-adrenoceptor stimulation enhances

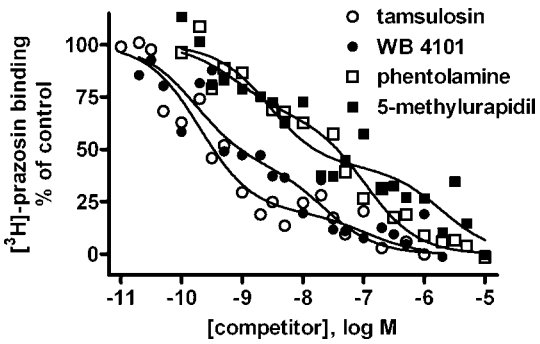

Figure 3 Competition of subtype-selective antagonists for [^{3}H]prazosin binding to human prostate membranes. Data are means of 4–5 experiments (error bars deleted for clarity). Modified with permission from Michel *et al.* (1996).

inositol phosphate formation, apparently *via* an α_{1A}-like subtype (Haynes & Hill, 1997). Experiments with rat prostatic neuroendocrine cells demonstrated α_1-adrenoceptor agonist-induced Ca^{2+} elevations, which were abolished by the phospholipase C inhibitor U 73,122 (Kim *et al.*, 2003). Since α_1-adrenoceptor stimulation can promote cellular growth in several cell types, it has been studied whether this is also the case in cultured prostatic smooth muscle cells. However, several studies with human tissue failed to detect growth modulation in these cells by α_1-adrenoceptor agonists or antagonists (Boesch *et al.*, 1999; Michel *et al.*, 2000b). Whether this reflects of lack of such responses in prostatic stromal cells or rather a loss of α_1-adrenoceptor expression under cell culture conditions (Ohmi *et al.*, 1999) has remained unclear. The latter possibility is supported by some reports demonstrating modulation of prostatic cell growth upon treatment with α_1-adrenoceptor agonists and antagonists *in vivo* (see below).

Most functional *in vitro* studies have focused on prostate contraction. This has been assessed by videoimaging of human prostatic stromal cell contractions in the presence of α_1-adrenoceptor agonists and antagonists in some cases (Corvin *et al.*, 1998), but the vast majority of studies has used classical organ bath approaches with prostatic strips from various species. α-Adrenoceptor-mediated contraction of the prostate of rabbits (Honda *et al.*, 1985), dogs (Felsen *et al.*, 1994; Delaflotte *et al.*, 1996) and humans (Hieble *et al.*, 1985; Lepor *et al.*, 1988b; Chapple *et al.*, 1989; Yu *et al.*, 1994) appears to occur largely, if not exclusively, *via* α_1- rather than α_2-adrenoceptors; such findings have been obtained by comparing the effects of α_1-selective agonists such as phenylephrine with those of α_2-selective agonists such as UK 14,304, or by comparing the antagonism of noradrenaline by α_1-selective antagonists such as prazosin with that of α_2-selective antagonists such as rauwolscine. The predominant, if not exclusive, mediation of prostate contraction *via* α_1- rather than α_2-adrenoceptors is further supported by a potent antagonism of field stimulation-induced contraction by prazosin (Tsujii *et al.*, 1992; Guh *et al.*, 1995). At least within the rat (Steidle *et al.*, 1989) and human prostate (Lepor *et al.*, 1994), all regions appear to have similar contractile responsiveness to α_1-adrenoceptor stimulation.

The identification of α_1- relative to α_2-adrenoceptors is classically based upon a high affinity of the antagonist prazosin for the former (see Bylund *et al.*, 1994). However, it has repeatedly been reported that prazosin has lower potency than expected (but, nevertheless, higher than that for α_2-adrenoceptors) for some α_1-adrenoceptor responses. Based upon such observations, it has been proposed to classify α_1-adrenoceptor responses with relatively low prazosin affinity as α_{1L} (Muramatsu *et al.*, 1990; Ohmura *et al.*, 1992); in this scheme, all three cloned α_1-adrenoceptor subtypes have high affinity for prazosin and are designated as α_{1H}. Despite extensive searches and completion of the sequencing of the human genome, a corresponding gene for the proposed α_{1L}-adrenoceptor has not been identified. Rather, it has been reported that transfection of cells that lack functional α_1-adrenoceptors with the cDNA encoding the α_{1A}-adrenoceptor can induce the presence of a receptor with relatively low prazosin affinity under some experimental conditions; on the other hand, prazosin low-affinity sites did not become detectable in cells transfected with α_{1B}- or α_{1D}-adrenoceptors (Ford *et al.*, 1997; Daniels *et al.*, 1999). Therefore, it is now generally assumed that the relatively low prazosin affinity observed in some settings represents a phenotype of the cloned α_{1A}-adrenoceptor rather than a distinct receptor subtype. Nevertheless, the α_{1L} phenomenon is important for the pharmacology of the prostate, since it affects not only prazosin but also other antagonists with a quinazoline structure, such as alfuzosin, doxazosin and terazosin, which are routinely used in BPH treatment; in contrast, antagonists from other chemical classes such as tamsulosin do not discriminate the classical α_{1A} pharmacology and its α_{1L} phenotype (Ford *et al.*, 1997; Daniels *et al.*, 1999). With regard to $\alpha_{1A/L}$-adrenoceptors in the prostate, most studies have been performed in rabbits and humans. Binding affinities of both prazosin and tamsulosin in human prostate membranes were consistent with their reported affinities at cloned α_{1A}-adrenoceptors (Figure 4), and studies in isolated human prostatic cells using fluorescent ligands have reported even higher affinities, possibly reflecting technical differences between mixed tissue and isolated cell approaches (MacKenzie *et al.*, 2000). On the other hand, the reported functional potencies of prazosin in the rabbit prostate were about 1.5 log units lower than its affinity in binding studies with cloned α_{1A}-adrenoceptors or in human prostate membranes (Figure 4). These data demonstrate that the α_1-adrenoceptor mediating contraction of the rabbit prostate has the characteristics of an α_{1L}-adrenoceptor. Similarly low prazosin potencies were found in functional studies with the canine prostate (range pA_2 7.90–8.55) (Ohmura *et al.*, 1993; Buckner *et al.*, 1996; Leonardi *et al.*, 1997) or rat prostate (pK_B 8.13–8.78) (Killam *et al.*, 1995; Homma *et al.*, 2000). On the other hand, tamsulosin has similar affinity for the classical α_{1A}-adrenoceptor and its α_{1L} phenotype in rabbit prostate (Figure 4), and this was confirmed in radioligand-binding studies with [^{3}H]prazosin in bovine prostate, where tamsulosin had a similarly high affinity at prazosin high- and low-affinity sites (pK_i 9.13 and 8.99, respectively) (Maruyama *et al.*, 1998). Other radioligand-binding studies suggested that the canine prostate expresses a much larger abundance of low- than high-affinity binding sites for [^{3}H]prazosin (Ohmura *et al.*, 1993). Studies in human prostate have yielded a less clear picture. Thus, the reported functional potencies of prazosin were only slightly lower than to be expected based upon the affinity estimates from the radioligand-binding studies, and higher than the functional estimates in the rabbit prostate (Figure 4).

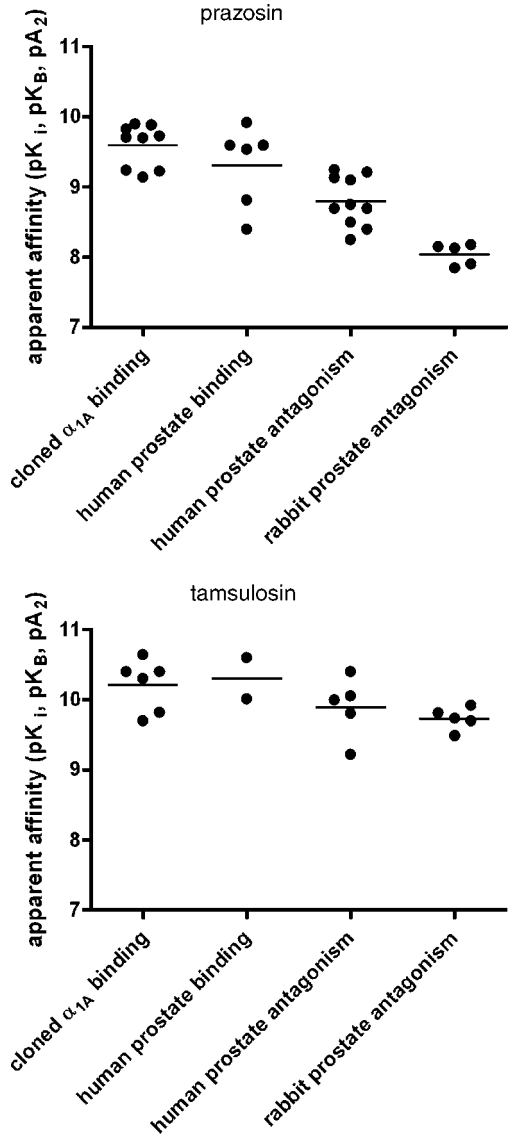

Figure 4 Potency and affinity of prazosin and tamsulosin at cloned α_{1A}-adrenoceptors and those in human and rabbit prostate. Data are from individual studies (filled circles) and their means (horizontal lines), and given as pK_i (binding studies) or pK_B/pA_2 (functional studies), as taken from the following references: Hieble *et al.* (1985); Chapple *et al.* (1989); Morita & Kondo (1992a); Yamada *et al.* (1992); Lefevre-Borg *et al.* (1993); Testa *et al.* (1993; 1996); Faure *et al.* (1994); Forray *et al.* (1994); Goetz *et al.* (1994); Teng *et al.* (1994); Yu *et al.* (1994); Hancock *et al.* (1995); Marshall *et al.* (1995); Tseng-Crank *et al.* (1995); Chueh *et al.* (1996); Ford *et al.* (1996); Hatano *et al.* (1996); Kenny *et al.* (1996); Michel *et al.* (1996); Leonardi *et al.* (1997); Martin *et al.* (1997); Noble *et al.* (1997); Chang *et al.* (2000).

Interestingly, functional affinity estimates (pA_2 or pK_B values) for other quinazolines in rabbit prostate (alfuzosin: median 7.22, range 7.19–7.25 (two studies), doxazosin: 7.01 (one study), terazosin: median 7.71, range 7.46–7.96 (two studies)) were also much lower than their pK_i values in binding studies with human prostate (alfuzosin: median 8.18, range 7.46–8.29 (four studies), doxazosin: median 8.40, range 8.20–8.60 (two studies), terazosin: median 8.52, range 7.96–8.98 (five studies)) or cloned α_{1A}-adrenoceptors (alfuzosin: median 8.20, range 7.99–8.42 (five studies), doxazosin: median 8.56, range 8.34–8.89 (five studies), terazosin: median 8.35, range 7.58–8.53 (seven studies)), whereas much smaller discrepancies were observed for human prostate (alfuzosin: not reported; doxazosin: median 8.32, range 8.20–8.43 (two studies), terazosin: median 7.60, range 7.38–8.48 (three studies)) (Hieble *et al.*, 1985; Chapple *et al.*, 1989; Morita & Kondo, 1992a; Yamada *et al.*, 1992; Lefevre-Borg *et al.*, 1993; Testa *et al.*, 1993; 1996; Faure *et al.*, 1994; Forray *et al.*, 1994; Goetz *et al.*, 1994; Teng *et al.*, 1994; Yu *et al.*, 1994; Hancock *et al.*, 1995; Marshall *et al.*, 1995; Tseng-Crank *et al.*, 1995; Chueh *et al.*, 1996; Ford *et al.*, 1996; Hatano *et al.*, 1996; Kenny *et al.*, 1996; Michel *et al.*, 1996; Leonardi *et al.*, 1997; Martin *et al.*, 1997; Noble *et al.*, 1997; Chang *et al.*, 2000). Therefore, the overall evidence suggests that the α_1-adrenoceptors mediating contraction of the rabbit and canine prostate have relatively low affinity for prazosin and other quinazolines; in contrast, the receptor mediating contraction of the human prostate has only moderately lower affinity for prazosin and other quinazolines than would be expected based upon their affinity in binding studies, and hence cannot be unequivocally classified as α_{1L}.

Numerous studies have investigated which of the cloned α_1-adrenoceptor subtypes mediates prostate contraction *in vitro*. Similar to the radioligand-binding studies in this tissue, the early studies have relied largely on the ability of the subtype-selectively alkylating chloroethylclonidine to identify receptor subtypes. Later studies have used panels of drugs with largely moderate subtype selectivity and correlated their potencies in the prostate with their affinities at the cloned receptor subtypes. Highly subtype-selective drugs (mostly selective for α_{1A}-adrenoceptors) were only used in the most recent studies and have enabled an unequivocal identification of the receptor subtype being involved.

A study comparing α_1-adrenoceptor-mediated prostate contraction in rats with those in dogs and humans reported only negligible contraction in rats despite a greater density of receptors than in dogs, and hence did not allow for identification of receptor subtypes (Lepor *et al.*, 1994). However, most other studies reported more substantial contractions of the rat prostate. Based upon a lack of effect of chloroethylclonidine (10 μM for 10 or 30 min at 37°C), it was proposed that rat prostate contraction in response to phenylephrine is mediated by an α_{1A}-adrenoceptor (Yazawa & Honda, 1993). Since 30 nM of the α_{1D}-selective BMY 7378 did not shift the concentration–response curve for rat prostate, it was proposed that this subtype may not be involved (Deng *et al.*, 1996). More definitive conclusions could be based upon highly α_{1A}-selective antagonists such as L-771,688 (also known as SNAP 6383) (Chang *et al.*, 2000) or SNAP 7915 (Lagu *et al.*, 2000), which had a subnanomolar K_B value in rat prostate. Moreover, the α_{1A}-selective agonist A-61603 was an effective contractile agent in rat prostate (Chang *et al.*, 2000; Lagu *et al.*, 2000). Therefore, it appears that contraction of rat prostate is mediated predominantly, if not exclusively, by an α_{1A}-adrenoceptor. Based upon limited data, that is, insensitivity towards chloroethylclonidine and high affinity for 5-methylurapidil, a similar situation has been proposed in guinea-pig prostate (Haynes & Hill, 1997).

As stated above, contraction of the rabbit prostate occurs *via* an α_1-adrenoceptor with relatively low prazosin affinity (Honda *et al.*, 1985; Hiraoka *et al.*, 1995; Delaflotte *et al.*, 1996; Leonardi *et al.*, 1997; Martin *et al.*, 1997). Since studies

with cloned receptors have detected such low-affinity states for prazosin only with the α_{1A}- but not with the α_{1B}- or α_{1D}-adrenoceptor (Ford *et al*., 1997), these data already provided indirect evidence for a predominant involvement of the α_{1A}-adrenoceptor. Several studies with the moderately α_{1A}-selective antagonist tamsulosin support this conclusion (Honda *et al*., 1985; Honda & Nakagawa, 1986; Yamagishi *et al*., 1996; Martin *et al*., 1997; Taguchi *et al*., 1997). One study reported only low affinity for the moderately α_{1A}-selective antagonists WB 4101 and 5-methylurapidil, but suggested that this might be due to their poor recognition of the α_{1L}-phenotype (Hiraoka *et al*., 1995). However, somewhat greater potencies for both antagonists (but not for prazosin) were reported in another study, which also found a low potency of BMY 7378 (Delaflotte *et al*., 1996). Based upon correlation of potencies of a panel of five drugs with those at cloned subtypes, this study proposed involvement of an α_{1A}-adrenoceptor. Based upon a similar approach with a panel of six drugs, the same conclusion was reached (Martin *et al*., 1997). Another report from the same laboratory, but with largely distinct authors, reported identical values for all six drugs (van der Graaf *et al*., 1997). More definitive conclusions can be based upon the high potency of highly α_{1A}-selective antagonists such as silodosin (formerly known as KMD 3213) (Yamagishi *et al*., 1996) or B8805–033 (Eltze *et al*., 2001), which also had high potency. Taken together, these data demonstrate that contraction of the rabbit prostate occurs *via* an α_{1A}-adrenoceptor with low affinity for prazosin.

As stated above, contraction of the canine prostate, similar to that of the rabbit, occurs *via* an α_1-adrenoceptor with relatively low prazosin potency (Ohmura *et al*., 1993; Buckner *et al*., 1996; Leonardi *et al*., 1997). With regard to the cloned subtypes being involved, initial studies in the canine prostate reported that chloroethylclonidine (10 μM for 30 min at 37°C) reduced phenylephrine-induced contraction by 53% (Lepor *et al*., 1994). On the other hand, several moderately subtype-selective antagonists had Schild slopes around unity in the canine prostate, indicating the involvement of a single subtype only (Hancock *et al*., 1995). In later studies from the same investigators, the potency of a panel of 11 antagonists correlated better with that at the cloned α_{1A}- than the other α_1-adrenoceptor subtypes; moreover, in such studies the α_{1A}-selective A-61603 was a full agonist for canine prostate contraction (Buckner *et al*., 1996). Other investigators reached a similar conclusion based upon a panel of 14 antagonists (Leonardi *et al*., 1997). Moreover, highly α_{1A}-selective antagonists such as SNAP 7915 (Lagu *et al*., 2000) and L-771,688 (Chang *et al*., 2000) also had high potency in the canine prostate. Therefore, contraction of the canine prostate also involves predominantly, if not exclusively, an α_{1A}-adrenoceptor.

Early studies into the α_1-adrenoceptor subtype mediating contraction of the human prostate were primarily based upon chloroethylclonidine (10–100 μM for 30 min at 37°C) and have reported 30–80% inhibition of agonist- or field stimulation-induced contraction (Lepor *et al*., 1993b; 1994; Teng *et al*., 1994; Guh *et al*., 1995; Marshall *et al*., 1995). While these experiments did not allow definitive conclusions, later experimental approaches have consistently supported the suggestion that the contraction of the human prostate occurs predominantly, if not exclusively, *via* an α_{1A}-adrenoceptor. This was based upon a high potency of moderately α_{1A}-selective drugs such as WB 4101, 5-methylurapidil, SB 216,469 and tamsulosin (Marshall *et al*., 1995; Chess-Williams *et al*., 1996; Chueh *et al*., 1996; Noble *et al*., 1997), and upon the correlation of the potency of panels of antagonists with those at cloned receptor subtypes (Forray *et al*., 1994; Marshall *et al*., 1995; 1996; Ford *et al*., 1996; Kenny *et al*., 1996; Testa *et al*., 1996). Most recently, highly α_{1A}-selective antagonists such as L 771,688 (Chang *et al*., 2000), SNAP 7915 (Lagu *et al*., 2000) or B 8805–033 (Eltze *et al*., 2001) have allowed definitive conclusions. They are in line with potent and effective agonism by α_{1A}-selective drugs such as A 61603 (Chang *et al*., 2000; Lagu *et al*., 2000). Taken together, the overall evidence demonstrates that contraction of prostate of rats, guinea-pigs, rabbits, dogs and humans in response to exogenous agonists occurs largely, if not exclusively, *via* the α_{1A}-subtype. Some studies in isolated human prostate have used field stimulation to release endogenous agonist (Yu *et al*., 1994; Guh *et al*., 1995; Chueh *et al*., 1996). Those studies unequivocally reported high potency for α_{1A}-selective antagonists and hence demonstrate that human prostate contraction not only in response to exogenous but also to endogenous release agonist occurs *via* this subtype.

In contrast to the range of subtyping reports, only few studies have addressed the possible underlying mechanisms. Thus, contractions induced by exogenous noradrenaline (Teng *et al*., 1994) and by endogenous agonist released by field stimulation (Guh *et al*., 1995; 1996; Haynes & Hill, 1997) were sensitive to Ca^{2+} entry blockers such as nifedipine. One study in rats found that the protein kinase C inhibitors calphostin C and bisindolylmaleimide I did not affect noradrenaline-induced contraction (Ramasamy *et al*., 2002).

In vivo *function* *In vivo* studies on prostate function have typically measured alterations of IUP. In the interpretation of such data, it has to be taken into account that this response is a composite measure of the contractile force developed by the urethra and the surrounding prostate (see also the section on urethra). However, the contribution of the prostate appears to dominate because phenylephrine-induced IUP elevations were about 80% smaller in prostate-ablated male, castrated male or female as compared to prostate-intact rats (Akiyama *et al*., 1999). The systemic administration of α_1-adrenoceptor agonists such as noradrenaline, adrenaline or phenylephrine has been shown to increase IUP in anaesthetized rats (Guilmard *et al*., 1996; Martin *et al*., 1997; Akiyama *et al*., 1999), anaesthetized cats (Lefevre-Borg *et al*., 1993) and anaesthetized dogs (Breslin *et al*., 1993; Kenny *et al*., 1994; 1996; Testa *et al*., 1997; Witte *et al*., 1997; 2002; Pulito *et al*., 2000; Eltze *et al*., 2001) and conscious dogs (Brune *et al*., 2002). Similar IUP elevations have also been produced by the systemic administration of α_{1A}-selective agonists such as A 61603 (Knepper *et al*., 1995). Agonist-induced IUP elevations were inhibited by various α_1-adrenoceptor antagonists, including the clinically used alfuzosin (Lefevre-Borg *et al*., 1993; Kenny *et al*., 1994; Guilmard *et al*., 1996; Martin *et al*., 1997; Testa *et al*., 1997; Akiyama *et al*., 1999), doxazosin (Kenny *et al*., 1994; 1996; Martin *et al*., 1997; Witte *et al*., 2002), tamsulosin (Breslin *et al*., 1993; Kenny *et al*., 1994; 1996; Martin *et al*., 1997; Testa *et al*., 1997; Akiyama *et al*., 1999; Pulito *et al*., 2000; Brune *et al*., 2002; Witte *et al*., 2002) and terazosin (Breslin *et al*., 1993; Kenny *et al*., 1994; Martin *et al*., 1997; Testa *et al*., 1997; Witte *et al*., 1997; 2002; Akiyama *et al*.,

1999; Brune *et al*., 2002). Similar inhibition was also obtained when IUP elevations had been induced by stimulation of the hypogastric nerves (Lefevre-Borg *et al*., 1993; Leonardi *et al*., 1997; Sato *et al*., 2001), indicating its physiological relevance.

Many studies have compared the effects of antagonists on IUP with those on blood pressure. Most such studies appear to have been designed with the primary aim of demonstrating that the drug manufactured by the sponsoring company is superior to the comparator drugs in this regard rather than with a genuine interest to better understand the adrenergic control of prostatic function. Nevertheless, it appears from the overall body of evidence that drugs with selectivity for α_{1A}-adrenoceptors produce less blood pressure lowering for any given level of antagonism of the IUP response (Kenny *et al*., 1996; Testa *et al*., 1997; Akiyama *et al*., 1999; Pulito *et al*., 2000; Eltze *et al*., 2001). Together with the numerous *in vitro* studies on prostate contraction (see above), these data clearly demonstrate that prostate contraction occurs mainly, if not exclusively, *via* an α_{1A}-adrenoceptor. In agreement with these animal data, it has been found that nonselective α_1-adrenoceptor antagonists (Martorana *et al*., 1997; Witjes *et al*., 1997), the $\alpha_{1A/D}$-selective tamsulosin (Abrams *et al*., 1998) and the α_{1A}-selective RO700004 (Blue *et al*., 2002) can lower bladder outlet resistance in patients *in vivo*. However, the overall effect of α_1-adrenoceptor antagonists on bladder outlet resistance in patients is small and detected only inconsistently (see Kortmann *et al*., 2003).

Based upon the role of α_1-adrenoceptors in the modulation of cell growth in many other tissues, this possibility has also been investigated in the prostate. Studies in rat have found induction of atypical prostatic hyperplasia upon chronic (26 days) treatment with the agonist phenylephrine; these effects were blocked by the α_{1A}-antagonist RS 100,329, but not by BMY 3738, cyclazosin or yohimbine (Marinese *et al*., 2003). Treatment of BPH patients has been reported to reduce smooth muscle myosin heavy chain mRNA expression relative to control or androgen-ablated patients (Lin *et al*., 2001). Other studies have found that treatment with doxazosin and other quinazoline α_1-adrenoceptor antagonists induces apoptosis in the human prostate (Kyprianou *et al*., 1998; Chon *et al*., 1999; Tahmatzopoulos & Kyprianou, 2004). However, the assessment of apoptosis was largely based upon the TUNEL assay, which is known to frequently yield false-positive results, and no alterations of overall prostate size were seen with doxazosin relative to placebo in a large multi-year study (McConnell *et al*., 2003). Therefore, it appears unlikely that therapeutic doses of α_1-adrenoceptor antagonists indeed modulate prostate growth in an appreciable manner.

Regulation of receptor expression and function An increase in α_1-adrenoceptors with age was reported for rabbit prostate, but failed to reach statistical significance (Kondo *et al*., 1992). Patients with diabetes suffer from greater BPH symptoms than those without (Michel *et al*., 2000a). If this would be α-adrenoceptor-related, an enhanced responsiveness of the receptors would be expected in diabetes. However, a study in rats with streptozotocin-induced diabetes reported the opposite, that is, a reduced potency and efficacy of noradrenaline to contract the prostate *in vitro*, and insulin treatment *in vivo* restored the maximum effects but not the agonist potency; the protein kinase C inhibitor calphostin C (but not bisindolylmaleimide I) restored the contractile effects of noradrenaline in diabetic rats (Ramasamy *et al*., 2002). The authors interpreted these findings to indicate that an increased protein kinase C activity in prostates from diabetic rats may impair α_1-adrenoceptor function; desensitization of α_1-adrenoceptor function by protein kinase C activation is well documented (Yang *et al*., 1998a).

Most studies on the regulation of prostatic α_1-adrenoceptor expression and function were performed in the context of BPH, which develops in an androgen-dependent manner. Accordingly, the effect of androgen removal on prostatic α_1-adrenoceptors has been studied in castrated rats. Castration causes a major shrinkage of the prostate. This was accompanied by a markedly loss of α_1-adrenoceptor-mediated elevations of IUP *in vivo* (Akiyama *et al*., 1999). *In vitro* studies found that strips from castrated rats developed a greater tension in response to α_1-adrenoceptor stimulation, but relative to KCl-induced contraction no change was observed (Homma *et al*., 2000), indicating that the enhanced responses are related to an altered overall tissue responsiveness and change in the glandular/stromal ratio. However, the potency of phenylephrine decreased in castrated rats. On the other hand, receptor expression at the protein level remained unchanged when expressed relative to tissue weight and even increased when expressed per mg protein, although α_{1A}-adrenoceptor mRNA expression was reduced (Homma *et al*., 2000). A similar receptor increase relative to protein content was also observed by others after castration (Takahashi *et al*., 2002).

In contrast to the rat studies, a seven-fold increase in total α_1-adrenoceptor mRNA, largely due to an increase in α_{1A}-adrenoceptor mRNA, was reported in the prostate from BPH patients (Nasu *et al*., 1996). A later study found numerically more α_{1A}-adrenoceptor mRNA in the urethral, central and peripheral areas of the prostate from BPH as compared to control patients, but the differences were statistically significant only for the central area (Figure 5) (Moriyama *et al*., 1998). While an increased receptor density was also found at the protein level in some studies (Morita & Kondo, 1992b; Kondo *et al*., 1993), others reported a similar density of α_1-adrenoceptors in the prostate from BPH and control patients (Figure 5) (Chapple *et al*., 1989; Tsujii *et al*., 1992), and another study found a similar density in prostates from men with symptomatic vs asymptomatic BPH (Gup *et al*., 1990). Accordingly, a fourth study reported a lack of correlation between the density of prostatic α_1-adrenoceptors and the size of the adenoma within a group of BPH patients (Kawabe *et al*., 1990). A report on increased contraction of prostatic tissue from BPH patients in response to phenylephrine does not necessarily contradict these findings, because that study found similarly increased contraction in response to prostaglandin E_2 and $F_{2\alpha}$ and also receptor-independently in response to KCl (Kitada & Kumazawa, 1987), suggesting that the enhanced responses may not be related to alterations in adrenoceptor expression, but rather to the increased contribution of smooth muscle cells in the hyperplastic human prostate. Moreover, another study found similar contractile responses in patients with symptomatic and asymptomatic BPH (Gup *et al*., 1989).

Since α-adrenoceptor antagonists are the most frequent form of rational medical BPH treatment, the effects of agonist and antagonist treatment on receptor expression have been studied. In cultured human prostatic smooth muscle cells, surprisingly only α_{1B}- and α_{1D}-, but no α_{1A}-, adrenoceptor

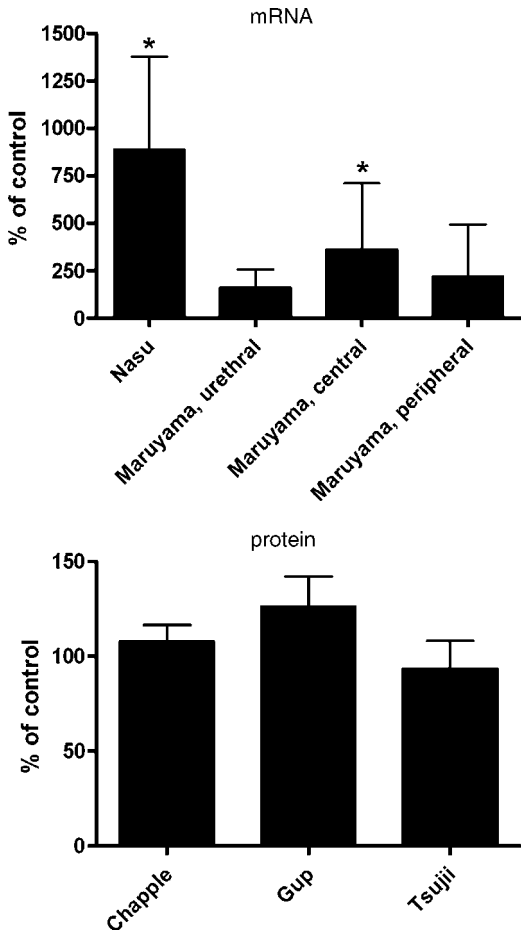

Figure 5 α_{1A}-Adrenoceptor mRNA (upper panel) and total α_1-adrenoceptor protein expression (lower panel) in prostates from BPH patients. Data are expressed as % of the corresponding control group and taken from Chapple *et al*. (1989); Gup *et al*. (1990); Tsujii *et al*. (1992); Nasu *et al*. (1996); Moriyama *et al*. (1998). $^*P<0.05$ vs control in the respective study. Note that Moriyama *et al*. have studied the urethral, central and peripheral zones in parallel.

mRNA was detected; neither expression was altered by treatment with the agonist phenylephrine or the antagonist doxazosin (Boesch *et al*., 1999). In *in vivo* studies, an 8-week treatment with doxazosin also failed to alter the expression of rat prostatic α_1-adrenoceptor subtype mRNA, and only a 12-week treatment with very high doxazosin doses (2 and 4 mg kg^{-1}) caused detectable upregulation (Foster *et al*., 2004). Given the inverse agonism of doxazosin (Hein *et al*., 2001) and the known regulation of all three subtypes by agonists (Yang *et al*., 1999), these findings are difficult to understand.

Clinical implications Based upon the above data, it is obvious that α_1-adrenoceptor antagonists are an option for the treatment of BPH, and indeed they have become the most widely used form of medical treatment worldwide (see Roehrborn & Schwinn, 2004). Original concepts have assumed that their beneficial effects are largely mediated by the α_1-adrenoceptors mediating prostate smooth muscle contraction, that is, α_{1A}-adrenoceptors. In this line of thought, α_{1A}-selective antagonists may be fully effective, but lack some of the side effects associated with blockade of the other subtypes, for example, in the vasculature (Rudner *et al*., 1999). While the excellent tolerability of the moderately α_{1A}-selective tamsulosin is in line with this reasoning, several lines of evidence have challenged this view. Firstly, alfuzosin, which has similar affinity for all α_1-adrenoceptor subtypes except for those in the α_{1L}-state, has a similarly good tolerability profile as tamsulosin (see Michel *et al*., 2001). Secondly, mechanistic models in which symptom relief in BPH patients is related to prostatic smooth muscle relaxation imply that α_1-adrenoceptor antagonists relieve BPH symptoms by lowering bladder outlet obstruction. While such effects have been found in some clinical studies, the overall evidence suggests that clinically used doses of α_1-adrenoceptor antagonists have little effect on bladder outlet obstruction (see Kortmann *et al*., 2003). Thirdly, and perhaps most importantly, α_1-adrenoceptor antagonists were also shown to improve typical BPH symptoms in animal models of bladder outlet obstructions under experimental conditions where a reduction of bladder outlet resistance was not possible (Broten *et al*., 1998; Gu *et al*., 2004). Taken together, these data suggest that mechanisms distinct from prostatic smooth muscle relaxation may contribute to the α_1-adrenoceptor antagonist-induced symptom relief in BPH patients. Candidates for such alternative mechanisms include α_1-adrenoceptors, possibly α_{1D}-adrenoceptors, in the bladder or in parts of the spinal cord involved in micturition control. However, the anatomical and pharmacological identity of such sites remains to be determined.

Another conceptually important point is the finding that some α_1-adrenoceptor antagonists, specifically alfuzosin and tamsulosin, have little effect on blood pressure at doses where they clearly relieve BPH symptoms (see Michel *et al*., 2001). Several not mutually exclusive theories have been proposed in this regard. One possibility is that all three α_1-adrenoceptor subtypes contribute to vascular resistance (see Guimaraes & Moura, 2001). This implies that no α_1-adrenoceptor antagonist could ever be fully free of vascular effects, but that subtype-selective antagonists may have fewer than others. Based upon the above, it appears that inhibition of α_{1A}- and perhaps also α_{1D}-adrenoceptors is sufficient for a full therapeutic response in BPH and hence a drug with little affinity for α_{1B}-adrenoceptors may have less effect on the vasculature. In this context, it should be considered that the relative role of α_{1B}-adrenoceptors increases in blood vessels of the elderly (Rudner *et al*., 1999). This might explain why tamsulosin has little vascular effects, but it cannot explain a relatively similar cardiovascular profile of alfuzosin that does not discriminate between α_1-adrenoceptor subtypes (Michel & Insel, 1994). A second theory relates to a possible differential tissue distribution of some α_1-adrenoceptor antagonists. Thus, it has been demonstrated that both alfuzosin and tamsulosin enrich in tissues of the lower urinary tract as compared to other tissues or the blood stream (Sato *et al*., 2001; Mottet *et al*., 2003; Romic *et al*., 2003). However, it remains unclear whether other drugs with greater blood pressure effects, for example, doxazosin or terazosin, lack such properties despite being chemically rather similar to alfuzosin. Thirdly, it has been proposed that other pharmacokinetic factors, that is, a late t_{max} and a small C_{max}/C_{trough} ratio, may contribute. Indeed, formulations fulfilling those criteria have been introduced for alfuzosin, doxazosin and tamsulosin, and in all three cases this has resulted in moderate improvements in tolerability (Kirby *et al*., 2001; Roehrborn *et al*., 2003;

Chapple *et al*., 2005; Michel *et al*., 2005a, b). While all of the above are plausible hypotheses, it must be emphasized that each remains to be proven and that they are not mutually exclusive.

α_2-*Adrenoceptors*

mRNA and protein expression Based upon PCR (Eason & Liggett, 1993) and RNase protection assays (Perälä *et al*., 1992), all three cloned α_2-adrenoceptor subtypes are expressed at the mRNA level in the human prostate. Studies at the protein level have sometimes been performed using immunohistochemistry (Slater *et al*., 2000) and receptor autoradiography (James *et al*., 1989; Felsen *et al*., 1994), but in most cases have relied upon radioligand-binding experiments with tissue homogenates. In young and old rats, α_{2A}-adrenoceptor immunoreactivity was mainly detected in the prostatic epithelium (Slater *et al*., 2000). Studies in canine prostates have reported a similar density of total α_2-adrenoceptors in the proximal, midportion and distal region (Shapiro *et al*., 1987), and on a histological level they were largely found in the epithelium (Felsen *et al*., 1994). Autoradiographic studies in the human prostate have localized the α_2-adrenoceptors mainly in association with blood vessels and, to a lesser extent, with the prostate epithelium (James *et al*., 1989). Competition radioligand-binding studies reported that prostatic α_2-adrenoceptors belong predominantly, if not exclusively, to the α_{2A}-subtype (Goepel *et al*., 1997). Several studies in the human prostate have compared the density of α_2- and α_1-adrenoceptor-binding sites. While some studies reported a similar abundance of both receptors at the protein level (Hedlund *et al*., 1985; Shapiro & Lepor, 1986; Lepor *et al*., 1988a, b; Gup *et al*., 1990; Morita & Kondo, 1992a, b), others reported significantly fewer α_2- than α_1-adrenoceptors (Chapple *et al*., 1989; James *et al*., 1989; Yamada *et al*., 1992); some studies also reported much fewer α_2- than α_1-adrenoceptors, but the difference did not reach statistical significance with small patient numbers (Kawabe *et al*., 1990; Hatano *et al*., 1996).

In vitro *and* in vivo *functions* Similar to most other tissues, α_2-adrenoceptor agonists and antagonists can inhibit or enhance, respectively, the pre-junctional noradrenaline release in the human prostate (Hedlund *et al*., 1985). Accordingly, the α_2-adrenoceptor agonist clonidine inhibited the field stimulation-induced contraction of the human prostate (Guh *et al*., 1995). In rat prostate neuroendocrine cells α_2-adrenoceptor agonists can inhibit high-voltage-operated Ca^{2+} channels in a pertussis toxin-sensitive manner (Kim *et al*., 2003), but the effect of this on the overall prostatic function remains unclear.

In line with the above studies demonstrating the presence of prostatic α_2-adrenoceptors in the epithelium rather than the stroma, however, no post-junctional contractile function of prostatic α_2-adrenoceptors has been identified, since they contribute little to α-adrenergic contraction in dogs (Somers *et al*., 1989; Felsen *et al*., 1994), horses (Garcia-Sacristan *et al*., 1984) or humans (Hedlund *et al*., 1985; Hieble *et al*., 1985; Lepor *et al*., 1988b; Chapple *et al*., 1989; Gup *et al*., 1989; Steidle *et al*., 1989; Hatano *et al*., 1996). In light of the α_2-adrenoceptor-mediated *in vitro* contraction of the rabbit urethra (see above) but not the prostate, *in vivo* data with systemic administration of α_2-adrenoceptor drugs on canine IUP are interpreted to represent effects upon the urethra rather than the prostate (Shapiro *et al*., 1987). One study has reported that, in a rat model of androgen/oestrogen-induced BPH, chronic administration of the α_2-agonist atipamezole increased prostatic compliance, while an α_1-agonist did not (Constantinou & Omata, 1996); unfortunately, no follow-up of this interesting study has been reported.

Regulation of receptor expression and function While no major alterations in α_{2A}-adrenoceptor immunoreactivity were found in histochemical studies comparing prostates from 12-week- and 18-month-old rats (Slater *et al*., 2000), another study comparing 6-month- and 4.5–5-year-old rabbits found a numerically higher overall α_2-adrenoceptor density in the older animals, but the difference failed to reach statistical significance (Kondo *et al*., 1992). Four studies in patients with BPH have reported an increased α_2-adrenoceptor density as compared to controls (Chapple *et al*., 1989; Gup *et al*., 1990; Morita & Kondo, 1992b; Kondo *et al*., 1993). While this finding seems remarkably consistent, its relevance remains unclear due to the lack of knowledge regarding physiological functions of this receptor in the prostate (apart from pre-junctional inhibition). For this very reason, α_2-adrenoceptors are not considered to represent a therapeutic target at present.

β-*Adrenoceptors*

mRNA and protein expression The presence of β-adrenoceptor subtype mRNA has been studied in a limited way only. One study using Northern blot analysis has demonstrated the presence of β_2-adrenoceptor mRNA in rats (Collins *et al*., 1988), and another study using RNase protection assays without previous PCR amplification has reported the presence of β_3-adrenoceptor mRNA in humans (Berkowitz *et al*., 1995).

The presence of prostatic β-adrenoceptor subtypes at the protein level has been studied by radioligand-binding and immunological techniques. Saturation-binding studies using [^{3}H]dihydroalprenolol or [^{125}I]iodocyanopindolol have reported 125–1000 fmol mg^{-1} protein in rats (Poyet *et al*., 1986b; Collins *et al*., 1988; Guthrie *et al*., 1990; Gousse *et al*., 1991; Fukumoto *et al*., 1993; Chen *et al*., 1995), 60 fmol mg^{-1} protein in pigs (Goepel *et al*., 1997) and 40–280 fmol mg^{-1} protein in humans (Tsujii *et al*., 1992; Goepel *et al*., 1997). Three of these studies have performed a characterization of the β-adrenoceptor subtypes being present using competition binding, and report a predominant contribution of the β_2-subtype in rats, pigs and humans (Poyet *et al*., 1986b; Gousse *et al*., 1991; Goepel *et al*., 1997). However, all of these studies have employed radioligand concentrations that are unlikely to detect β_3-adrenoceptors (Hoffmann *et al*., 2004). This did not allow detection of possibly present β_3-adrenoceptors and may underestimate the total β-adrenoceptor density. Indeed, immunohistochemical studies with a selective antibody have detected the presence of β_1- and β_3-adrenoceptors in the rat and human prostate, respectively (Chamberlain *et al*., 1999; Slater *et al*., 2000).

In vitro *and* in vivo *functions* The functional presence of β-adrenoceptors in the rat, guinea-pig and human prostate has been demonstrated by adenylyl cyclase stimulation studies, largely involving β_2-adrenoceptors (Shima *et al*., 1980; Poyet *et al*., 1986a; Purvis *et al*., 1986; Solano *et al*., 1994; Carmena

et al., 1995; 1997; Chen *et al*., 1995; Haynes & Hill, 1997; Juarranz *et al*., 1998). While β-adrenoceptor stimulation did not inhibit α_1-adrenoceptor-mediated inositol phosphate formation (Haynes & Hill, 1997) and did not alter basal prostatic tone (Caine *et al*., 1975), it has been shown to inhibit α_1-adrenoceptor-mediated, field stimulation-induced or receptor-independent prostate contraction in rats (Kalodimos & Ventura, 2001), guinea-pigs (Haynes & Hill, 1997), dogs (Normandin & Lodge, 1996), horses (Garcia-Sacristan *et al*., 1984) and humans (Tsujii *et al*., 1992; Drescher *et al*., 1994). Based upon subtype-selective agonists and antagonists, the relaxation responses against field stimulation and against α_1-adrenoceptor agonist were reported to occur *via* different receptors in guinea-pigs, that is, *via* β_1-adrenoceptors for field stimulation and *via* β_2-adrenoceptors for the α_1-response (Haynes & Hill, 1997). In rat prostate, the nonselective agonists isoprenaline and adrenaline, the β_1-selective noradrenaline and RO363, and the β_2-selective salbutamol relaxed field stimulation-induced contraction, whereas the β_3-selective BRL 37,344 did not; on the other hand, the β_2-antagonist ICI 118,551 antagonized responses to isoprenaline and salbutamol, while the β_1-antagonist atenolol did not (Kalodimos & Ventura, 2001). Relaxation of field stimulation-induced contraction affected the nifedipine-sensitive component only, whereas relaxation of the α_1-response inhibited the nifedipine-sensitive and -insensitive components of contraction (Haynes & Hill, 1997). Since the relaxations were mimicked by the adenylyl cyclase stimulator forskolin and by phosphodiesterase inhibitors in various species (Tsujii *et al*., 1992; Drescher *et al*., 1994; Chen *et al*., 1995; Normandin & Lodge, 1996; Kalodimos & Ventura, 2001), these appear to be cAMP-mediated. β-Adrenoceptor stimulation has also been reported to regulate gene transcription in the rat prostate (Guthrie *et al*., 1990). Some functional data also demonstrate the presence of β-adrenoceptors, most likely β_2-adrenoceptors, in the LNCaP human prostate cancer cell line (Nagmani *et al*., 2003). To the best of our knowledge, *in vivo* studies on the β-adrenoceptor-mediated regulation of prostatic function have not been reported, apart from the above-mentioned studies on IUP, which may partly reflect prostatic effects.

Regulation of receptor expression and function A possible regulation of the prostatic β-adrenoceptor expression and function by age has been studied in rats (Chen *et al*., 1995). The number of [^{3}H]dihydroalprenolol-binding sites increased somewhat between 4 and 12 weeks of age and remained at a similar level up to an age of 52 weeks. In contrast, isoprenaline-stimulated adenylyl cyclase activity increased stronger between the ages of 2 and 12 weeks, and then continuously declined until the age of 104 weeks. Concomitantly, the ratio of G_s/G_i α-subunits, as assessed by toxin-catalyzed ADP ribosylation, also was lowest at 2 weeks, maximum at 12 weeks, and declined at 52 and 104 weeks. On the other hand, an immunohistochemical study has reported an increased β_1-adrenoceptor density in 20- vs 3-month-old rats (Slater *et al*., 2000). Thus, a reduced prostatic β-adrenoceptor function in immature and old rats appears to reflect predominantly a change in G-protein function rather than one in receptor expression. Accordingly, a study in rabbits did not detect any alterations of β-adrenoceptor-binding sites with age (Kondo *et al*., 1992).

A study in tissue specimens from control patients and those with BPH has found a significant reduction of β-adrenoceptor, assessed as [^{3}H]dihydroalprenolol-binding sites, by about 40% in the latter group (Tsujii *et al*., 1992). This was accompanied by a loss of isoprenaline-induced (but not forskolin-induced) prostate relaxation; concomitantly, propranolol enhanced noradrenaline-induced contraction in control, but not in BPH tissue. Together, these data suggest that a reduced receptor density is the cause of an impaired β-adrenoceptor-mediated relaxation in BPH.

Since the prostate is an androgen-dependent tissue, the modulation of prostatic β-adrenoceptor expression and function by androgens has been studied in rats. While castration reduced prostatic β-adrenoceptor density at the protein level and testosterone replacement restored it, castration had little effect on β_2-adrenoceptor mRNA and testosterone replacement had only transient increasing effects (Collins *et al*., 1988). Upregulation of β_2-adrenoceptor mRNA by androgen has also been observed in the intact rat prostate (Guthrie *et al*., 1990). The functional relevance of this is supported by the observation that castration reduced β-adrenoceptor-mediated adenylyl cyclase stimulation in the prostate, and this was also restored by androgen replacement; basal and forskolin-stimulated adenylyl cyclase activity exhibited a similar androgen-dependent pattern (Poyet *et al*., 1986a; Purvis *et al*., 1986). Thus, β-adrenoceptor expression and function in the prostate are androgen-dependent, and this may involve transcriptional and post-transcriptional effects at the receptor level, as well as receptor-independent effect.

Three studies have evaluated the effect of diabetes on prostatic β-adrenoceptors using the rat streptozotocin model. The initial report described a marked reduction in prostatic β-adrenoceptors in diabetes (shown to be a predominant β_2-adrenoceptor population), which was fully restored upon insulin substitution (Gousse *et al*., 1991). A later study from the same group confirmed these findings, and reported that such regulation was not accompanied by alterations of the relative contribution of agonist high- and low-affinity sites (Fukumoto *et al*., 1993). Other investigators found that diabetes markedly reduced isoprenaline- or forskolin-stimulated adenylyl cyclase activity in rat prostate, but this was not corrected by insulin treatment; in that study, diabetes was also associated with a reduced expression of G_s, $G_{i1/2}$ and $G_{i3/o}$ proteins, which also was not fully restored by insulin treatment (Carmena *et al*., 1997). Finally, it has also been reported that chronic alcohol intake increases isoprenaline-induced adenylyl cyclase activity as well as expression of G_s and $G_{i1/2}$ proteins in rat prostate (Juarranz *et al*., 1998).

Clinical implications Taken together, these data demonstrate that all three β-adrenoceptor subtypes appear to be expressed in the prostate at the mRNA and protein levels, but precise quantitative information on their relative contribution is lacking. β-Adrenoceptor stimulation in the prostate causes smooth muscle relaxation, an effect involving both β_1- and β_2-adrenoceptors; while a single study in rats argues against an involvement of β_3-adrenoceptors, the present data are too limited to allow definitive conclusions. Ageing, ambient androgen levels and diabetes can regulate prostatic β-adrenoceptor expression and function.

Conclusions

In conclusion, adrenoceptors in the lower urinary tract predominantly mediate continence-supporting functions, that is, a relaxation of smooth muscle in the detrusor *via* β-adrenoceptors and hence increased bladder compliance and a contraction of smooth muscle in the bladder neck, urethra and prostate *via* α_1-adrenoceptors. In humans, the former appears to involve mainly β_3-adrenoceptors, and the latter mainly α_{1A}-adrenoceptors. α_2-Adrenoceptors, possibly from the α_{2A}-subtype, mediate pre-junctional inhibition of transmitter release, but the great abundance of this subtype makes other, as yet undefined functions likely. Most of the six other adrenoceptor subtypes are also expressed to varying extents in the lower urinary tract, but their function remains unclear. In pathophysiological settings, particularly during states of bladder outlet obstruction in elderly men with enlarged prostates, α_1-adrenoceptors may contribute to the symptoms and hence antagonists of these receptors effectively relieve such symptoms. β_3-Adrenoceptor agonists may be a useful form of treatment of bladder dysfunction in the context of OAB, but perhaps also in the context of BPH. The latter possibility makes the possible combination of α_1-adrenoceptor antagonist with β_3-adrenoceptor agonist treatment potentially interesting.

Work in our lab on the subject of this article has been funded in part by the Deutsche Forschungsgemeinschaft, Astellas and Boehringer Ingelheim. We thank Dr A.G. Ramage for his help with the section on central nervous α_1-adrenoceptors.

References

ABRAMS, P., CARDOZO, L., FALL, M., GRIFFITHS, D., ROSIER, P., ULMSTEN, U., VAN KERREBROECK, P., VICTOR, A. & WEIN, A. (2002). The standardisation of terminology of lower urinary tract function: report from the standardisation sub-committee of the International Continence Society. *Neurourol. Urodyn.,* **21,** 167–178.

ABRAMS, P., TAMMELA, T.L., HELLSTRÖM, P., MARBERGER, M., DJAVAN, B.R., LUNGLMAYR, G., PANOS, P. & LAVAL, K.-U. (1998). European pressure-flow investigation of tamsulosin in men with lower urinary tract symptoms (LUTS) suggestive of benign prostatic obstruction (BPO) – ESPRIT study. *J. Urol.,* **159,** 256.

AKIYAMA, K., HORA, M., TATEMICHI, S., MASUDA, N., NAKAMURA, S., YAMAGISHI, R. & KITAZAWA, M. (1999). KMD-3213, a uroselective and long-acting a_{1a}-adrenoceptor antagonist, tested in a novel rat model. *J. Pharmacol. Exp. Ther.,* **291,** 81–91.

ANDERSSON, K.-E. & WEIN, A.J. (2004). Pharmacology of the lower urinary tract: basis for current and future treatments of urinary incontinence. *Pharmacol. Rev.,* **56,** 581–631.

ANDERSSON, K.-E., LARSSON, B. & SJOGREN, C. (1984). Characterization of α-adrenoceptors in the female rabbit urethra. *Br. J. Pharmacol.,* **81,** 293–300.

BADAWI, J.K., UECELEHAN, H., HATZINGER, M., MICHEL, M.S., HAFERKAMP, A. & BROSS, S. (2005). Relaxant effects of b-adrenergic agonists on porcine and human detrusor muscle. *Acta Physiol. Scand.,* **185,** 151–159.

BAKER, J.G. (2005). The selectivity of b-adrenoceptor antagonists at the human β1, β2 and β3 adrenoceptors. *Br. J. Pharmacol.,* **141,** 317–322.

BANNOWSKY, A. & JUENEMANN, K.-P. (2003). Innervation and function of the female urinary bladder and urethra. *EAU Update Series,* **1,** 120–127.

BARTSCH, G., MÜLLER, H.R., OBERHOLZER, M. & ROHR, H.P. (1979). Light microscopic stereological analysis of the normal human prostate and of benign prostatic hyperplasia. *J. Urol.,* **122,** 487–491.

BERGES, R.R., PIENTKA, L., HÖFNER, K., SENGE, T. & JONAS, U. (2001). Male lower urinary tract symptoms and related health care seeking in Germany. *Eur. Urol.,* **39,** 682–687.

BERKOWITZ, D.E., NARDONE, N.A., SMILEY, R.M., PRICE, D.T., KREUTTER, D.K., FREMEAU, R.T. & SCHWINN, D.A. (1995). Distribution of β_3-adrenoceptor mRNA in human tissues. *Eur. J. Pharmacol.,* **289,** 223–228.

BING, W., CHANG, S., HYPOLITE, J.A., DISANTO, M.E., ZDERIC, S.A., ROLF, L., WEIN, A.J. & CHACKO, S. (2003). Obstruction-induced changes in urinary bladder smooth muscle contractility: a role for rho kinase. *Am. J. Physiol.,* **285,** F990–F997.

BLUE, D., ZINNER, N., GRINO, P., GABLES, C., CRAGER, M. & FORD, A.P.D.W. (2002). RO700004, a selective α_{1A}-adrenoceptor antagonist, does not improve lower urinary tract symptoms in men with benign prostatic hyperplasia. *J. Urol.,* **167** (Suppl.), 265.

BLUE, D.R., DANIELS, D.V., GEVER, J.R., JETT, M.F., O'YANG, C., TANG, H.M., WILL, T.J. & FORD, A.P.D.W. (2004). Pharmacological characteristics of Ro 115–1240, a selective $\alpha_{1A/1L}$-adrenoceptor agonist: a potential therapy of stress urinary incontinence. *BJU Int.,* **93,** 162–170.

BOESCH, S.T., CORVIN, S., ZHANG, J., ROGATSCH, H., BARTSCH, G. & KLOCKER, H. (1999). Modulation of the differentiation status of cultured prostatic smooth muscle cells by an α1-adrenergic receptor antagonist. *Prostate,* **39,** 226–233.

BOLLE, P., FIDANZA, S. & TUCCI, P. (1999). Response to isoprenaline of rabbit detrusor muscle following exposure to 5, 8, 11, 14 eicosatetraenoic acid. Role of prostanoids on beta-adrenergic-evoked response. *J. Auton. Pharmacol.,* **19,** 161–165.

BRAHMADEVARA, N., SHAW, A.M. & MACDONALD, A. (2003). Evidence against β_3-adrenoceptors or low affinity state of β_2-adrenoceptors mediating relaxation in rat isolated aorta. *Br. J. Pharmacol.,* **138,** 99–106.

BRESLIN, D., FIELDS, D.W., CHOU, T.-C., MARION, D.N., KANE, M., VAUGHAN JR, E.D. & FELSEN, D. (1993). Medical management of benign prostatic hyperplasia: a canine model comparing the *in vivo* efficacy of alpha-1 adrenergic antagonists in the prostate. *J. Urol.,* **149,** 395–399.

BROTEN, T., SCOTT, A., SIEGL, P.K.S., FORRAY, C., LAGU, B., NAGARATHNAM, D., WONG, W.C., MARZABADI, M., MURALI DHAR, T.G. & GLUCHOWSKI, C. (1998). Alpha-1 adrenoceptor blockade inhibits detrusor instability in rats with bladder outlet obstruction. *FASEB J.,* **12,** A445.

BRUNE, M.E., KATWALA, S.P., MILICIC, I., WITTE, D.G., KERWIN JR, J.F., MEYER, M.D., HANCOCK, A.A. & WILLIAMS, M. (2002). Effect of fiduxosin, an antagonist selective for α_{1A}- and α_{1D}-adrenoceptors, on intraurethral and arterial pressure responses in conscious dogs. *J. Pharmacol. Exp. Ther.,* **300,** 487–494.

BUCKNER, S.A., OHEIM, K.W., MORSE, P.A., KNEPPER, S.M. & HANCOCK, A.A. (1996). α_1-Adrenoceptor-induced contractility in rat aorta is mediated by the α_{1D} subtype. *Eur. J. Pharmacol.,* **297,** 241–248.

BYLUND, D.B., EIKENBERG, D.C., HIEBLE, J.P., LANGER, S.Z., LEFKOWITZ, R.J., MINNEMAN, K.P., MOLINOFF, P.B., RUFFOLO JR, R.R. & TRENDELENBURG, U. (1994). IV. International Union of Pharmacology Nomenclature of Adrenoceptors. *Pharmacol. Rev.,* **46,** 121–136.

CAINE, M., RAZ, S. & ZEIGLER, M. (1975). Adrenergic and cholinergic receptors in the human prostate, prostatic capsule and bladder neck. *Br. J. Urol.,* **47,** 193–202.

CARMENA, M.J., CLEMENTE, C., CARRERO, I., SOLANO, R.M. & PRIETO, J.C. (1997). G-proteins and β-adrenergic stimulation of adenylate cyclase activity in the diabetic rat prostate. *Prostate,* **33,** 46–54.

CARMENA, M.J., GARCIA-PARAMIO, P., SOLANO, R.M. & PRIETO, J.C. (1995). Protein kinase C regulation of the adenylyl cyclase system in rat prostatic epithelium. *Prostate,* **27,** 204–211.

CHAMBERLAIN, P.D., JENNINGS, K.H., PAUL, F., CORDELL, J., HOLMES, S.D., PARK, J., CHAMBERS, J., SENNITT, M.V., STOCK, M.J., CAWTHORNE, M.A., YOUNG, P.W. & MURPHY, G.J. (1999). The tissue distribution of the human β_3-adrenoceptor studied using a monoclonal antibody: direct evidence of the β_3-adrenoceptor in human adipose tissue, atrium and skeletal muscle. *Int. J. Obesity,* **23,** 1057–1065.

CHANG, D.J., CHANG, T.K., YAMANISHI, S.S., SALAZAR, F.H.R., KOSAKA, A.H., KHARE, R., BHAKTA, S., JASPER, J.R., SHIEH, I.-S., LESNICK, J.D., FORD, A.P.D.W., DANIELS, D.V., EGLEN, R.M., CLARKE, D.E., BACH, C. & CHAN, H.W. (1998). Molecular cloning, genomic characterization and expression of novel human α_{1A}-adrenoceptor isoforms. *FEBS Lett.,* **422,** 279–283.

CHANG, R.S.L., CHEN, T.-B., O'MALLEY, S.S., PETTIBONE, D.J., DISALVO, J., FRANCIS, B., BOCK, M.G., FREIDINGER, R., NAGARATHNAM, D., MIAO, S.W., SHEN, Q., LAGU, B., DHAR, T.G.M., TYAGARAJAN, S., MARZABADI, M.R., WONG, W.C., GLUCHOWSKI, C. & FORRAY, C. (2000). *In vitro* studies on L-771, 688 (SNAP 6383), a new potent and selective α_{1A}-adrenoceptor antagonist. *Eur. J. Pharmacol.,* **409,** 301–312.

CHAPPLE, C.R., AL-SHUKRI, S.H., GATTEGNO, B., HOLMES, S., MARTINEZ-SAGARRA, J.M., SCARPA, R.M., VAN VIERSSEN TRIP, O.B., VIK, V. & VAN DER PUTTEN-SLOB, I. (2005). Tamsulosin oral controlled absorption system (OCAS) in patients with lower urinary tract symptoms suggestive of benign prostatic hyperplasia (LUTS/BPH): efficacy and tolerability in a placebo and active comparator controlled phase 3a study. *Eur. Urol. Suppl.,* **4,** 33–44.

CHAPPLE, C.R., AUBRY, M.L., JAMES, S., GREENGRASS, P.M., BURNSTOCK, G., TURNER-WARWICK, R.T., MILROY, E.J.G. & DAVEY, M.J. (1989). Characterisation of human prostatic adrenoceptors using pharmacology receptor binding and localisation. *Br. J. Urol.,* **63,** 487–496.

CHEN, C., ISHIKAWA, Y., AMANO, I., EGUCHI, T. & ISHIDA, H. (1995). Age-dependent changes in response of rat prostatic tissues to isoproterenol and forskolin: changes with sexual maturation in function of G proteins. *Mech. Ageing Dev.,* **81,** 1–13.

CHEN, Q., TAKAHASHI, S., ZHONG, S., HOSODA, C., ZHENG, H.-Y., OGUSHI, T., FUJIMURA, T., OHTA, N., TANOUE, A., TSUJIMOTO, G. & KITAMURA, T. (2005). Function of the lower urinary tract in mice lacking α_{1d}-adrenoceptor. *J. Urol.,* **174,** 370–374.

CHESS-WILLIAMS, R., CHAPPLE, C.R., VERFÜRTH, F., NOBLE, A.J., COULDWELL, C.J. & MICHEL, M.C. (1996). The effects of SB 216469, an antagonist which discriminates between the α_{1A}-adrenoceptor and the human prostatic α_1-adrenoceptor. *Br. J. Pharmacol.,* **119,** 1093–1100.

CHON, J.K., BORKOWSKI, A., PARTIN, A.W., ISAACS, J.T., JACOBS, S.C. & KYPRIANOU, N. (1999). α_1-Adrenoceptor antagonists terazosin and doxazosin induce prostate apoptosis without affecting cell proliferation in patients with benign prostatic hyperplasia. *J. Urol.,* **161,** 2002–2008.

CHOPRA, B., BARRICK, S.R., MEYERS, S., BECKEL, J.M., ZEIDEL, M.L., FORD, A.P.D.W., DE GROAT, W.C. & BIRDER, L.A. (2005). Expression and function of bradykinin B1 and B2 receptors in normal and inflamed rat urinary bladder urothelium. *J. Physiol. (London),* **562,** 859–871.

CHUEH, S.-C., GUH, J.-H., CHEN, J., LAI, M.-K., KO, F.-N. & TENG, C.-M. (1996). Inhibition by tamsulosin of tension responses of human hyperplastic prostate to electrical field stimulation. *Eur. J. Pharmacol.,* **305,** 177–180.

COLLINS, S., QUARMBY, V.E., FRENCH, F.S., LEFKOWITZ, R.J. & CARON, M.G. (1988). Regulation of the β_2-adrenergic receptor and its mRNA in the rat ventral prostate by testosterone. *FEBS Lett.,* **233,** 173–176.

CONLEY, R.K., WILLIAMS, T.J., FORD, A.P.D.W. & RAMAGE, A.G. (2001). The role of α_1-adrenoceptors and 5-HT_{1A} receptors in the control of the micturition reflex in male anaesthetized rats. *Br. J. Pharmacol.,* **133,** 61–72.

CONSTANTINOU, C.E. & OMATA, S. (1996). Analysis of the relative biomechanical effects of alpha 1 and alpha 2 antagonists in modifying the compliance of the prostate and micturition parameters of the hormonally manipulated male rat. *Neurourol. Urodyn.,* **15,** 85–101.

CORVIN, S., BÖSCH, S.T., EDER, I., THURNHER, M., BARTSCH, G. & KLOCKER, H. (1998). Videoimaging of prostatic stromal-cell contraction: an *in vitro* model for studying drug effects. *Prostate,* **37,** 209–214.

DANIELS, D.V., GEVER, J.R., JASPER, J.R., KAVA, M.S., LESNICK, J.D., MELOY, T.D., STEPAN, G., WILLIAMS, T.J., CLARKE, D.E., CHANG, D.J. & FORD, A.P.D.W. (1999). Human cloned α_{1A}-adrenoceptor isoforms display α_{1L}-adrenoceptor pharmacology in functional studies. *Eur. J. Pharmacol.,* **370,** 337–343.

DANUSER, H. & THOR, K.B. (1995). Inhibition of central sympathetic and somatic outflow to the lower urinary tract of the cat by the α_1-adrenergic receptor antagonist prazosin. *J. Urol.,* **153,** 1308–1312.

DAS, A.K., LEGGETT, R.E., WHITBECK, C., EAGEN, G. & LEVIN, R.M. (2002). Effect of doxazosin on rat urinary bladder function after partial outlet obstruction. *Neurourol. Urodyn.,* **21,** 160–166.

DELAFLOTTE, S., AUGUET, M. & CHABRIER, P.E. (1996). Pharmacological evidence that different α_1 adrenoceptor subtypes mediate contraction in rabbit prostate and hypogastric artery. *Acta Physiol. Scand.,* **158,** 241–251.

DENG, X.F., CHEMTOB, S. & VARMA, D.R. (1996). Characterization of α_{1D}-adrenoceptor subtype in rat myocardium, aorta and other tissues. *Br. J. Pharmacol.,* **119,** 269–276.

DEPLANNE, V. & GALZIN, A.M. (1996). Functional characterization of alpha-1-adrenoceptor subtypes in the prostatic urethra and trigone of male rabbit. *J. Pharmacol. Exp. Ther.,* **278,** 527–534.

DEPLANNE, V., PALEA, S. & ANGEL, I. (1998). The adrenergic, cholinergic and NANC nerve-mediated contractions of the female rabbit bladder neck and proximal, medial and distal urethra. *Br. J. Pharmacol.,* **123,** 1517–1524.

DERWEESH, I.H., WHEELER, M.A. & WEISS, R.M. (2000). Alterations in G-proteins and β-adrenergic responsive adenylyl cyclase in rat urinary bladder during ageing. *J. Pharmacol. Exp. Ther.,* **294,** 969–974.

DRESCHER, P., ECKERT, R.E. & MADSEN, P.O. (1994). Smooth muscle contractility in prostatic hyperplasia: role of cyclic adenosine monophosphate. *Prostate,* **25,** 76–80.

DURANT, P.A., LUCAS, P.C. & YAKSH, T.L. (1988). Micturition in the unanaesthetized rat: spinal vs peripheral pharmacology of the adrenergic system. *J. Pharmacol. Exp. Ther.,* **245,** 426–435.

EASON, M.G. & LIGGETT, S.B. (1993). Human α_2-adrenergic receptor subtype distribution: widespread and subtype-selective expression of α_2C10, α_2C4, and α_2C2 mRNA in multiple tissues. *Mol. Pharmacol.,* **44,** 70–75.

ELTZE, M., BOER, R., MICHEL, M.C., HEIN, P., TESTA, R., ULRICH, W.-R., KOLASSA, N. & SANDERS, K.H. (2001). *In vitro* and *in vivo* uroselectivity of B8805–033, an antagonist with high affinity at prostatic α_{1A}- vs α_{1B}- and α_{1D}-adrenoceptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **363,** 649–662.

FAURE, C., PIMOULE, C., VALLANCIEN, G., LANGER, S.Z. & GRAHAM, D. (1994). Identification of α_1-adrenoceptor subtypes present in the human prostate. *Life Sci.,* **54,** 1595–1605.

FELSEN, D., ERNSBERGER, P., SUTARIA, P.M., NEJAT, R.J., NGUYEN, P., MAY, M., BRESLIN, D.S., MARION, D.N. & VAUGHAN JR, E.D. (1994). Identification, localization and functional analysis of imidazoline and alpha adrenergic receptors in canine prostate. *J. Pharmacol. Exp. Ther.,* **268,** 1063–1071.

FORD, A.P.D.W., ARREDONDO, N.F., BLUE, D.R., BONHAUS, D.W., JASPER, J.R., KAVA, M.S., LESNICK, J., PFISTER, J.R., SHIEH, I.A., VIMONT, R.L., WILLIAMS, T.J., MCNEAL, J.E., STAMEY, T.A. & CLARKE, D.E. (1996). RS-17053 (*N*-[2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-a, a-dimethyl-1H-indole-3-ethanamine hydrochloride), a selective α_{1A}-adrenoceptor antagonist, displays low affinity for functional α_{1A}-adrenoceptors in human prostate: implications for adrenoceptor classification. *Mol. Pharmacol.,* **49,** 209–215.

FORD, A.P.D.W., DANIELS, D.V., CHANG, D.J., GEVER, J.R., JASPER, J.R., LESNICK, J.D. & CLARKE, D.E. (1997). Pharmacological pleiotropism of the human recombinant α_{1A}-adrenoceptor: implications for α_1-adrenoceptor classification. *Br. J. Pharmacol.,* **121,** 1127–1135.

FORRAY, C., BARD, J.A., WETZEL, J.M., CHIU, G., SHAPIRO, E., TANG, R., LEPOR, H., HARTIG, P.R., WEINSHANK, R.L., BRANCHEK, T.A. & GLUCHOWSKI, C. (1994). The α_1-adrenergic receptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned human α_{1C} subtype. *Mol. Pharmacol.,* **45,** 703–708.

FOSTER JR, H.E., YONO, M., SHIN, D., TAKAHASHI, W., POURESMAIL, M., AFIATPOUR, P. & LATIFPOUR, J. (2004). Effects of chronic administration of doxazosin on α_1-adrenoceptors in the rat prostate. *J. Urol.,* **172,** 2465–2470.

FRAZIER, E.P., MATHY, M.-J., PETERS, S.L.M. & MICHEL, M.C. (2005a). Does cyclic AMP mediate rat urinary bladder relaxation by isoproterenol? *J. Pharmacol. Exp. Ther.,* **313,** 260–267.

FRAZIER, E.P., SCHNEIDER, T. & MICHEL, M.C. (2005b). β-Adrenergic urinary bladder relaxation in rats prone to overactive bladder. *FASEB J.,* **19,** A535.

FUJIMURA, T., TAMURA, K., TSUTSUMI, T., YAMAMOTO, T., NAKAMURA, K., KOIBUCHI, Y., KOBAYASHI, M. & YAMAGUCHI, O. (1999). Expression and possible functional role of the β3-adrenoceptor in human and rat detrusor muscle. *J. Urol.,* **161,** 680–685.

FUKUMOTO, Y., YOSHIDA, M., DOKITA, S., KAMAI, T., WEISS, R.M. & LATIFPOUR, J. (1993). The reversal effect of insulin on diabetes-induced alterations in beta adrenergic and muscarinic receptors in rat prostate. *J. Urol.,* **149,** 1602–1607.

GARCIA-SACRISTAN, A., CASANUEVA, C.R., CASTILLA, C. & LABADIA, A. (1984). Adrenergic receptors in the urethra and prostate of the horse. *Res. Vet. Sci.,* **36,** 57–60.

GILLESPIE, J.I. (2004). Noradrenaline inhibits autonomic activity in the isolated guinea pig bladder. *BJU Int.,* **93,** 401–409.

GOEPEL, M., ERDBRÜGGER, W., WITTMANN, A. & MICHEL, M.C. (1996). Atypical affinities of ICI 118, 551 at porcine β_2-adrenoceptors. *Br. J. Pharmacol.,* **117** (Suppl.), 198.

GOEPEL, M., WITTMANN, A., RÜBBEN, H. & MICHEL, M.C. (1997). Comparison of adrenoceptor subtype expression in porcine and human bladder and prostate. *Urol. Res.,* **25,** 199–206.

GOETZ, A.S., LUTZ, M.W., RIMELE, T.J. & SAUSSY JR, D.L. (1994). Charactarization of alpha-1 adrenoceptor subtypes in human and canine prostate membranes. *J. Pharmacol. Exp. Ther.,* **271,** 1228–1233.

GOPALAKRISHNAN, M., WHITEAKER, K.L., MOLINARI, E.J., DAVIS-TABER, R., SCOTT, V.E.S., SHIEH, C.-C., BUCKNER, S.A., MILICIC, I., CAIN, J.C., POSTL, S., SULLIVAN, J.P. & BRIONI, J.D. (1999). Characterization of the ATP-sensitive potassium channels (K_{ATP}) expressed in guinea pig bladder smooth muscle cells. *J. Pharmacol. Exp. Ther.,* **289,** 551–558.

GOUSSE, A., YOSHIDA, M., WEISS, R.M. & LATIPOUR, J. (1991). Beta adrenergic receptor alterations in diabetic rat prostate: effects of insulin and dietary myoinositol. *Prostate,* **19,** 121–131.

GRUNEBERGER, A. (1984). Treatment of motor urge incontinence with clenbuterol and flavoxate hydrochloride. *Br. J. Obstetr. Gynaecol.,* **91,** 275–278.

GU, B., REITER, J.P., SCHWINN, D.A., SMITH, M.P., KORSTANJE, C., THOR, K.B. & DOLBER, P.C. (2004). Effects of α_1-adrenergic receptor subtype selective antagonists on lower urinary tract function in rats with bladder outlet obstruction. *J. Urol.,* **172,** 758–762.

GUH, J.-H., CHUEH, S.-C., KO, F.-N. & TENG, C.-M. (1995). Characterization of α_1-adrenoceptor subtypes in tension response of human prostate to electrical field stimulation. *Br. J. Pharmacol.,* **115,** 142–146.

GUH, J.-H., KO, F.-N., CHUEH, S.-C., LAI, M.-K. & TENG, C.-M. (1996). Ouabain-induced increases in resting tone of human hyperplastic prostate following repeated noradrenaline and electrical field stimulation. *Br. J. Pharmacol.,* **117,** 1716–1720.

GUILMARD, C., AUGUET, M. & CHABRIER, P.E. (1996). Pharmacological characterization of alpha$_1$-adrenoceptor subtype mediating regulation of arterial pressure and urethral perfusion pressure in the anaesthetized rat. *J. Auton. Pharmacol.,* **16,** 197–203.

GUIMARAES, S. & MOURA, D. (2001). Vascular adrenoceptors: an update. *Pharmacol. Rev.,* **53,** 319–356.

GUP, D.I., SHAPIRO, E., BAUMANN, M. & LEPOR, H. (1989). Contractile properties of human prostate adenomas and the development of infravesical obstruction. *Prostate,* **15,** 105–114.

GUP, D.I., SHAPIRO, E., BAUMANN, M. & LEPOR, H. (1990). Autonomic receptors in human prostate adenomas. *J. Urol.,* **143,** 179–185.

GUTHRIE, P.D., FREEMAN, M.R., LIAO, S.T. & CHUNG, L.W. (1990). Regulation of gene expression in rat prostate by androgen and β-adrenergic receptor pathways. *Mol. Endocrinol.,* **4,** 1343–1353.

HAMPEL, C., ARTIBANI, W., ESPUNA PONS, M., HAAB, F., JACKSON, S., ROMERO, J., GAVART, S. & PAPANICOLAOU, S. (2004). Understanding the burden of stress urinary incontinence in Europe: a qualitative review of the literature. *Eur. Urol.,* **46,** 15–27.

HAMPEL, C., DOLBER, P.C., SMITH, M.P., SAVIC, D.L., THÜROFF, J.W., THOR, K.B. & SCHWINN, D.A. (2002). Modulation of bladder α1-adrenergic receptor subtype expression by bladder outlet obstruction. *J. Urol.,* **167,** 1513–1521.

HANCOCK, A.A., BUCKNER, S.A., IRELAND, L.M., KNEPPER, S.M. & KERWIN JR, J.F. (1995). Actions of terazosin and its enantiomers at subtypes of α_1- and α_2-adrenoceptors. *J. Receptor Signal Transd. Res.,* **15,** 863–885.

HARADA, T. & CONSTANTINOU, C.E. (1993). The effect of alpha$_2$ agonists and antagonists on the lower urinary tract of the rat. *J. Urol.,* **149,** 159–164.

HATANO, A., TANG, R., WALDEN, P.D. & LEPOR, H. (1996). The α-adrenoceptor antagonist properties of the enantiomers of doxazosin in the human prostate. *Eur. J. Pharmacol.,* **313,** 135–143.

HAYNES, J.M. & HILL, S.J. (1997). β-Adrenoceptor-mediated inhibition of α_1-adrenoceptor-mediated and field stimulation-induced contractile responses in the prostate of the guinea pig. *Br. J. Pharmacol.,* **122,** 1067–1074.

HEDLUND, H., ANDERSSON, K.-E. & LARSSON, B. (1985). Alpha-adrenoceptors and muscarinic receptors in the isolated human prostate. *J. Urol.,* **134,** 1291–1298.

HEIN, P., GOEPEL, M., COTECCHIA, S. & MICHEL, M.C. (2001). A quantitative analysis of antagonism and inverse agonism at wild-type and constitutively active hamster α_{1B}-adrenoceptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **363,** 34–39.

HIEBLE, J.P., BYLUND, D.B., CLARKE, D.E., EIKENBURG, D.C., LANGER, S.Z., LEFKOWITZ, R.J., MINNEMAN, K.P. & RUFFOLO JR, R.R. (1995). International Union of Pharmacology X. Recommendation for nomenclature of α_1-adrenoceptors: consensus update. *Pharmacol. Rev.,* **47,** 267–270.

HIEBLE, J.P., CAINE, M. & ZALAZNIK, E. (1985). *In vitro* characterization of the α-adrenoceptors in human prostate. *Eur. J. Pharmacol.,* **107,** 111–117.

HIRAOKA, Y., OHMURA, T., SAKAMOTO, S., HAYASHI, H. & MURAMATSU, I. (1995). Identification of α_1-adrenoceptor subtypes in the rabbit prostate. *J. Auton. Pharmacol.,* **15,** 271–278.

HIRASAWA, A., HORIE, K., TANAKA, T., TAKAGAKI, K., MURAI, M., YANO, J. & TSUJIMOTO, G. (1993). Cloning, functional expression and tissue distribution of human cDNA for the α_{1C}-adrenergic receptor. *Biochem. Biophys. Res. Commun.,* **195,** 902–909.

HIRASAWA, A., SHIBATA, K., HORIE, K., TAKEI, Y., OBIKA, K., TANAKA, T., MURAMOTO, N., TAKAGAKI, K., YANO, J. & TSUJIMOTO, G. (1995). Cloning, functional expression and tissue distribution of human α_{1C}-adrenoceptor splice variants. *FEBS Lett.,* **363,** 256–260.

HOFFMANN, C., LEITZ, M.R., OBERDORF-MAASS, S., LOHSE, M.J. & KLOTZ, K.-N. (2004). Comparative pharmacology of human β-adrenergic receptor subtypes – characterization of stably transfected receptors in CHO cells. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **369,** 151–159.

HOMMA, Y., HAMADA, K., NAKAYAMA, Y., TSUJIMOTO, G. & KAWABE, K. (2000). Effect of castration on contraction and α_1-adrenoceptor expression in rat prostate. *Br. J. Pharmacol.,* **131,** 1454–1460.

HONDA, K. & NAKAGAWA, C. (1986). Alpha-1 adrenoceptor antagonist effects of the optical isomers of YM-12617 in rabbit lower urinary tract and prostate. *J. Pharmacol. Exp. Ther.,* **239,** 512–516.

HONDA, K., MIYATA-OSAWA, A. & TAKENAKA, T. (1985). α_1-Adrenoceptor subtype mediating contraction of the smooth muscle in the lower urinary tract and prostate of rabbits. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **330,** 16–21.

HORINOUCHI, T. & KOIKE, K. (2001). Agonist activity of SR59230A at atypical β-adrenoceptors in guinea pig gastric fundus and duodenum. *Eur. J. Pharmacol.,* **416,** 165–168.

HORINOUCHI, T., TANAKA, Y. & KOIKE, K. (2003). Evidence for the primary role for 4-aminopyridine-sensitive K_V channels in β_3-adrenoceptor-mediated, cyclic AMP-independent relaxations of guinea-pig gastrointestinal smooth muscle. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **367,** 193–203.

HUDMAN, D., ELLIOTT, R.A. & NORMAN, R.I. (2000). Inhibition of the contractile response of the rat detrusor muscle by the β_2-adrenoceptor agonist clenbuterol. *Eur. J. Pharmacol.,* **392,** 79–85.

HUDMAN, D., ELLIOTT, R.A., WHITAKKER, P., TERRY, T.R., SANDHU, D.P. & NORMAN, R.I. (2001). Inhibition of the contractile responses of isolated human and rat bladders by clenbuterol. *J. Urol.,* **166,** 1969–1973.

IGAWA, Y., YAMAZAKI, Y., TAKEDA, H., AKAHANE, M., AJISAWA, Y., YONEYAMA, T. & NISHIZAWA, O. (1998). Possible β3-adrenoceptor-mediated relaxation of the human detrusor. *Acta Physiol. Scand.,* **164,** 117–118.

IGAWA, Y., YAMAZAKI, Y., TAKEDA, H., HAYAKAWA, K., AKAHANE, M., AJISAWA, Y., YONEYAMA, T., NISHIZAWA, O. & ANDERSSON, K.-E. (1999). Functional and molecular biological evidence for a possible β_3-adrenoceptor in the human detrusor muscle. *Br. J. Pharmacol.,* **126,** 819–825.

IGAWA, Y., YAMAZAKI, Y., TAKEDA, H., KAIDOH, K., AKAHANE, M., AJISAWA, Y., YONEYAMA, T., NISHIZAWA, O. & ANDERSSON, K.-E. (2001). Relaxant effects of isoproterenol and selective β3-adrenoceptor agonists on normal, low compliant and hyperreflexic human bladders. *J. Urol.,* **165,** 240–244.

INCI, K., ISMAILOGLU, U.B., SAHIN, A., SUNUR, A. & SAHIN-ERDEMLI, I. (2003). The effect of inflammation on rat urinary bladder-dependent relaxation in coaxial bioassay system. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **367,** 547–552.

ISHIZUKA, O., MATTIASSON, A. & ANDERSSON, K.-E. (1996a). Role of spinal and peripheral alpha$_2$ adrenoceptors in micturition in normal conscious rats. *J. Urol.,* **156,** 1853–1857.

ISHIZUKA, O., PERSSON, K., MATTIASSON, A., NAYLOR, A.M., WYLLIE, M.G. & ANDERSSON, K.-E. (1996b). Micturition in conscious rats with and without bladder outlet obstruction: role of spinal α_1-adrenoceptors. *Br. J. Pharmacol.,* **117,** 962–966.

JAMES, S., CHAPPLE, C.R., PHILLIPS, M.I., GREENGRASS, P.M., DAVEY, M.J., TURNER-WARWICK, R.T., MILROY, E.J.G. & BURNSTOCK, G. (1989). Autoradiographic analysis of alpha-adrenoceptors and muscarinic cholinergic receptors in the hyperplastic human prostate. *J. Urol.,* **142,** 438–444.

JEONG, M.S. & LEE, J.G. (2000). The role of spinal and peripheral α_1- and α_2-adrenoceptors on bladder activity induced by bladder distension and anaesthetized rat. *BJU Int.,* **85,** 925–931.

JOSEPH, S.S., COLLEDGE, W.H. & KAUMANN, A.J. (2004). Aspartate138 is required for the high-affinity ligand binding site but not for the low-affinity binding site of the β_1-adrenoceptor. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **370,** 223–226.

JOSEPH, S.S., LYNHAM, J.A., MOLENAAR, P., GRACE, A.A., COLLEDGE, W.H. & KAUMANN, A.J. (2003). Intrinsic sympathomimetic activity of (−)-pindolol mediated through a (−)-propranolol-resistant site of the β_1-adrenoceptor in human atrium and recombinant receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **368,** 496–503.

JUARRANZ, M.G., GUIJARRO, L.G., BODEGA, G. & PRIETO, J.C. (1998). G-Protein regulation of adenylate cyclase activity in rat prostatic membranes after chronic ethanol ingestion. *Prostate,* **36,** 226–234.

KAIDOH, K., IGAWA, Y., TAKEDA, H., YAMAZAKI, Y., AKAHANE, S., MIYATA, H., AJISAWA, Y., NISHIZAWA, O. & ANDERSSON, K.-E. (2002). Effects of selective β_2 and β_3-adrenoceptor agonists in detrusor hyperreflexia in conscious cerebral infarcted rats. *J. Urol.,* **168,** 1247–1252.

KALODIMOS, P.J. & VENTURA, S. (2001). β_2-Adrenoceptor-mediated inhibition of field stimulation induced contractile responses of the smooth muscle of the rat prostate gland. *Eur. J. Pharmacol.,* **431,** 81–89.

KAVA, M.S., BLUE JR, D.R., VIMONT, R.L., CLARKE, D.E. & FORD, A.P.D.W. (1998). α_{1L}-Adrenoceptor mediation of smooth muscle contraction in rabbit bladder neck: a model for lower urinary tract tissues of man. *Br. J. Pharmacol.,* **123,** 1359–1366.

KAWABE, K., MORIYAMA, N., HAMADA, K. & ISHIMA, T. (1990). Density and localization of alpha$_1$-adrenoceptors in hypertrophied prostate. *J. Urol.,* **143,** 592–595.

KEAST, J.R., KAWATANI, M. & DE GROAT, W.C. (1990). Sympathetic modulation of cholinergic transmission in cat vesical ganglia is mediated by α_1- and α_2-adrenoceptors. *Am. J. Physiol.,* **258,** R44–R50.

KENNY, B.A., MILLER, A.M., WILLIAMSON, I.J.R., O'CONNELL, J., CHALMERS, D.H. & NAYLOR, A.M. (1996). Evaluation of the pharmacological selectivity profile of α_1 adrenoceptor antagonists at prostatic α_1 adrenoceptors: binding, functional and *in vivo* studies. *Br. J. Pharmacol.,* **118,** 871–878.

KENNY, B.A., READ, A.M., NAYLOR, A.M., GREENGRASS, P.M., CARTER, A.J. & WYLLIE, M.G. (1994). Effect of alpha$_1$ adrenoceptor antagonists on prostatic pressure and blood pressure in the anesthetized dog. *Urology,* **44,** 52–57.

KILLAM, A.L., WATTS, S.W. & COHEN, M.L. (1995). Role of α_1-adrenoceptors and 5-HT$_2$ receptors in serotonin-induced contraction of rat prostate: autoradiographical and functional studies. *Eur. J. Pharmacol.,* **273,** 7–14.

KIM, J.H., SHIN, S.Y., NAM, J.H., HONG, E.-K., CHUNG, Y.-S., JEONG, J.Y., KANG, J., UHM, D.-Y. & KIM, S.J. (2003). Adrenergic regulation of the intracellular [Ca^{2+}] and voltage-operated Ca^{2+} channel currents in the rat prostate neuroendocrine cells. *Prostate,* **57,** 99–110.

KIRBY, R.S., ANDERSEN, M., GRATZKE, P., DAHLSTRAND, C. & HOYE, K. (2001). A combined analysis of double-blind trials of the efficacy and tolerability of doxazosin-gastrointestinal therapeutic system, doxazosin standard and placebo in patients with benign prostatic hyperplasia. *BJU Int.,* **87,** 192–200.

KITADA, S. & KUMAZAWA, J. (1987). Pharmacological characteristics of smooth muscle in benign prostatic hyperplasia and normal prostatic tissue. *J. Urol.,* **138,** 158–160.

KLAUS, S., MUZZIN, P., REVELLI, J.-P., CAWTHORNE, M.A., GIACOBINO, J.-P. & RICQUIER, D. (1995). Control of β_3-adrenergic receptor gene expression in brown adipocytes in culture. *Mol. Cell. Endocrinol.,* **109,** 189–195.

KNEPPER, S.M., BUCKNER, S.A., BRUNE, M.E., DEBERNARDIS, J.F., MEYER, M.D. & HANCOCK, A.A. (1995). A-61603, a potent α1-adrenergic receptor agonist, selective for the α1A receptor subtype. *J. Pharmacol. Exp. Ther.,* **274,** 97–103.

KOBAYASHI, H., ADACHI-AKAHANE, S. & NAGAO, T. (2000). Involvement of BK_{Ca} channels in the relaxation of detrusor muscle *via* β-adrenoceptors. *Eur. J. Pharmacol.,* **404,** 231–238.

KOBAYASHI, H., MIWA, T., NAGAO, T. & ADACHI-AKAHANE, S. (2003). Negative modulation of L-type Ca^{2+} channels *via* β-adrenoceptor stimulation in guinea-pig detrusor smooth muscle cells. *Eur. J. Pharmacol.,* **470,** 9–15.

KOLTA, M.G., WALLACE, L.J. & GERALD, M.C. (1984). Age-related changes in sensitivity of rat urinary bladder to autonomic agents. *Mech. Ageing Dev.,* **27,** 183–188.

KONDO, S., TASHIMA, Y. & MORITA, T. (1992). Adenergic and cholinergic muscarinic receptors in the prostate of young and old rabbits. *Urol. Int.,* **49,** 201–205.

KONDO, S., TASHIMA, Y. & MORITA, T. (1993). Quantitative analysis of adrenergic alpha-1 and alpha-2 receptors in human prostatic urethral tissue. *Br. J. Urol.,* **72,** 68–73.

KONTANI, H., TSUJI, T. & KIMURA, S. (2000). Effects of adrenergic α_2-receptor agonists on urinary bladder contraction in conscious rats. *Jpn. J. Pharmacol.,* **84,** 381–390.

KORTMANN, B.B.M., FLORATOS, D.L., KIEMENEY, L.A.L.M., WIJKSTRA, H. & DE LA ROSETTE, J.J.M.C.H. (2003). Urodynamic effects of alpha-adrenoceptor blockers: a review of clinical trials. *Urology,* **62,** 1–9.

KUBOTA, Y., NAKAHARA, T., YUNOKI, M., MITANI, A., MARUKO, T., SAKAMOTO, K. & ISHII, K. (2002). Inhibitory mechanism of BRL37344 on muscarinic receptor-mediated contractions of the rat urinary bladder smooth muscle. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **366,** 198–203.

KUNISAWA, Y., KAWABE, K., NIIJIMA, T., HONDA, K. & TAKENAKA, T. (1985). A pharmacological study of alpha adrenergic receptor subtypes in smooth muscle of human urinary bladder base and prostatic urethra. *J. Urol.,* **134,** 396–398.

KYPRIANOU, N., LITVAK, J.P., BORKOWSKI, A. & JACOBS, S.C. (1998). Induction of prostate apoptosis by doxazosin in benign prostatic hyperplasia. *J. Urol.,* **159,** 1810–1815.

LAGU, B., TIAN, D., JEON, Y., LI, C., WETZEL, J.M., NAGARATHNAM, D., SHEN, Q., FORRAY, C., CHANG, R.S.L., BROTEN, T.P., RANSOM, R.W., CHAN, T.-B., O'MALLEY, S.S., SCHORN, T.W., RODRIGUES, A.D., KASSAHUN, K., PETTIBONE, D.J., FREIDINGER, R. & GLUCHOWSKI, C. (2000). *De novo* design of a novel oxazolidinone analogue as a potent and selective α_{1A} adrenergic receptor antagonist with high oral bioavailability. *J. Med. Chem.,* **43,** 2775–2778.

LARSSON, B., SJÖGREN, C. & ANDERSSON, K.-E. (1986). Regional distribution of α-adrenoceptor subtypes in the female rabbit urethra. *Acta Physiol. Scand.,* **126,** 39–43.

LATIFPOUR, J., KONDO, S., O'HOLLAREN, B., MORITA, T. & WEISS, R.M. (1990). Autonomic receptors in urinary tract: sex and age differences. *J. Pharmacol. Exp. Ther.,* **253,** 661–667.

LEBLAIS, V., POURAGEAUD, F., IVORRA, M.D., MARTHAN, R. & MULLER, B. (2005). Comparison of the α-adrenoceptor-mediated effects of β_3-adrenoceptor ligands in rat pulmonary artery. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **371,** 535–539.

LECCI, A., GIULIANI, S., TRAMONTANA, M., SANTICIOLI, P., CRISCUOLI, M., DION, S. & MAGGI, C.A. (1998). Bladder distension and activation of the efferent function of sensory fibres: similarities with the effect of capsaicin. *Br. J. Pharmacol.,* **124,** 259–266.

LEFEVRE-BORG, F., O'CONNOR, S.E., SCHOEMAKER, H., HICKS, P.E., LECHAIRE, J., GAUTIER, E., PIERRE, F., PIMOULE, C., MANOURY, P. & LANGER, S.Z. (1993). Alfuzosin, a selective α_1-adrenoceptor antagonist in the lower urinary tract. *Br. J. Pharmacol.,* **109,** 1282–1289.

LEI, B., MORRIS, D.P., SMITH, M.P., SVETKEY, L.P., NEWMAN, M.F., ROTTER, J.I., BUCHANAN, T.A., BECKSTROM-STERNBERG, S.M., GREEN, E.D. & SCHWINN, D.A. (2005). Novel human α_{1a}-adrenoceptor single nucleotide polymorphisms alter receptor pharmacology and biological function. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **371,** 229–239.

LEINEWEBER, K., BÜSCHER, R., BRUCK, H. & BRODDE, O.-E. (2004). Adrenoceptor polymorphisms. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **369,** 1–22.

LEONARDI, A., HIEBLE, J.P., GUARNERI, L., NASELSKY, D.P., POGGESI, E., SIRONI, G., SULPIZIO, A.C. & TESTA, R. (1997). Pharmacological characterization of the uroselective alpha-1 antagonist Rec 15/2739 (SB 216469): role of the alpha-1L adrenoceptor in tissue selectivity, part I. *J. Pharmacol. Exp. Ther.,* **281,** 1272–1283.

LEPOR, H., BAUMANN, M. & SHAPIRO, E. (1988a). The alpha adrenergic binding properties of terazosin in the human prostate adenoma. *J. Urol.,* **140,** 664–667.

LEPOR, H., GUP, D.I., BAUMANN, M. & SHAPIRO, E. (1988b). Laboratory assessment of terazosin and alpha-1 blockade in prostatic hyperplasia. *Urology,* **32** (Suppl), 21–26.

LEPOR, H., TANG, R. & SHAPIRO, E. (1993b). The alpha-adrenoceptor subtype mediating the tension of human prostatic smooth muscle. *Prostate,* **22,** 301–307.

LEPOR, H., TANG, R., MERETYK, S. & SHAPIRO, E. (1993a). Binding and functional properties of $alpha_1$ adrenoceptors in different regions of the human prostate. *J. Urol.,* **150,** 253–256.

LEPOR, H., ZHANG, W., KOBAYASHI, S., TANG, R., WANG, B. & SHAPIRO, E. (1994). A comparison of the binding and functional properties of alpha-1 adrenoceptors and area density of smooth muscle in the human, canine and rat prostate. *J. Pharmacol. Exp. Ther.,* **270,** 722–727.

LEVIN, R.M., RUGGIERI, M.R. & WEIN, A.J. (1988). Identification of receptor subtypes in the rabbit and human urinary bladder by selective radio-ligand binding. *J. Urol.,* **139,** 844–848.

LI, G., LI, K., LI, Z. & WANG, P. (2003). Age-dependent changes in β-adrenoceptor function in human detrusor and possible mechanisms. *Chin. Med. J.,* **116,** 1511–1514.

LI, J.H., YASAY, G.D. & KAU, S.T. (1992). β-Adrenoceptor subtypes in the detrusor of guinea-pig urinary bladder. *Pharmacology,* **44,** 13–18.

LIN, V.K., BENAIM, E.A. & MCCONNELL, J.D. (2001). Alpha-blockade downregulates myosin heavy chain gene expression in human benign prostatic hyperplasia. *Urology,* **57,** 170–175.

LINDHOLM, P. & LOSE, G. (1986). Terbutaline (Bricanyl) in the treatment of female urge incontinence. *Urol. Int.,* **41,** 158–160.

LLUEL, P., DEPLANNE, V., HEUDES, D., BRUNEVAL, P. & PALEA, S. (2003a). Age-related changes in urethrovesical coordination in male rats: relationship with bladder instability? *Am. J. Physiol.,* **284,** R1287–R1295.

LLUEL, P., PALEA, S., BARRAS, M., GRANDADAM, F., HEUDES, D., BRUNEVAL, P., CORMAN, B. & MARTIN, D.J. (2000). Functional and morphological modifications of the urinary bladder in aging female rats. *Am. J. Physiol.,* **278,** R964–R972.

LLUEL, P., SALEA, S., RBIERE, P., BARRAS, M., TEILLET, L. & CORMAN, B. (2003b). Increased adrenergic contractility and decreased mRNA expression of NOS III in aging rat urinary bladders. *Fundam. Clin. Pharmacol.,* **17,** 633–641.

LONGHURST, P.A. & LEVENDUSKY, M. (1999). Pharmacological characterization of β-adrenoceptors mediating relaxation of the rat urinary bladder *in vitro*. *Br. J. Pharmacol.,* **127,** 1744–1750.

MA, F.H., HIGASHIRA-HOSHI, H. & ITOH, Y. (2002). Functional muscarinic M_2 and M_3 receptors and β-adrenoceptors in cultured rat bladder smooth muscle. *Life Sci.,* **70,** 1159–1172.

MACKENZIE, J.F., DALY, C.J., PEDIANI, J.D. & MCGRATH, J.C. (2000). Quantitative imaging in live human cells reveals intracellular α_1-adrenoceptor ligand-binding sites. *J. Pharmacol. Exp. Ther.,* **294,** 434–443.

MAGGI, C.A., SANTICIOLI, P., FURIO, M. & MELI, A. (1985). Dual effects of clonidine on micturition reflex in urethane anesthetized rats. *J. Pharmacol. Exp. Ther.,* **235,** 528–536.

MALLOY, B.J., PRICE, D.T., PRICE, R.R., BIENSTOCK, A.M., DOLE, M.K., FUNK, B.L., RUDNER, X.L., RICHARDSON, C.D., DONATUCCI, C.F. & SCHWINN, D.A. (1998). α1-Adrenergic receptor subtypes in human detrusor. *J. Urol.,* **160,** 937–943.

MALYSZ, J., BUCKNER, S.A., DAZA, A.V., MILICIC, I., PEREZ-MEDRANO, A. & GOPALAKRISHNAN, M. (2004). Functional characterization of large conductance calcium-activated K^+ channel openers in bladder and vascular smooth muscle. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **369,** 481–489.

MARINESE, D., PATEL, R. & WALDEN, P.D. (2003). Mechanistic investigation of the adrenergic induction of ventral prostate hyperplasia in mice. *Prostate,* **54,** 230–237.

MARSHALL, I., BURT, R.P. & CHAPPLE, C.R. (1995). Noradrenaline contractions of human prostate mediated by α_{1A}- (α_{1c}-) adrenoceptor subtype. *Br. J. Pharmacol.,* **115,** 781–786.

MARSHALL, I., BURT, R.P., GREEN, G.M., HUSSAIN, M.B. & CHAPPLE, C.R. (1996). Different subtypes of α_{1A}-adrenoceptor mediating contraction of rat epididymal vas deferens, rat hepatic portal vein and human prostate distinguished by the antagonist RS 17053. *Br. J. Pharmacol.,* **119,** 407–415.

MARTIN, D.J., LLUEL, P., GUILLOT, E., COSTE, A., JAMMES, D. & ANGEL, I. (1997). Comparative alpha-1 adrenoceptor subtype selectivity and functional uroselectivity of alpha-1 adrenoceptor antagonists. *J. Pharmacol. Exp. Ther.,* **282,** 228–235.

MARTORANA, G., GIBERTI, C., DI SILVERIO, F., VON HELAND, M., RIGATTI, P., COLOMBO, R., CASADEI, G. & PACIFICO, P. (1997). Effects of short-term treatment with the α_1-blocker alfuzosin on urodynamic pressure/flow parameters in patients with benign prostatic hyperplasia. *Eur. Urol.,* **32,** 47–53.

MARUYAMA, K., NAKAMURA, T., YOSHIHARA, T., FUKUTOMI, J., SUGIYAMA, K., HATTORIM, K., OHNUKI, T., WATANABE, K. & NAGATOMO, T. (1998). Tamsulosin: assessment of affinity of ^{3}H-prazosin bindings to two α_1-adrenoceptor subtypes (α_{1H} and α_{1L}) in bovine prostate and rat heart and brain. *Gen. Pharmacol.,* **31,** 597–600.

MATSUBARA, S., OKADA, H., SHIRAKAWA, T., GOTOH, A., KUNO, T. & KAMIDONO, S. (2002). Estrogen levels influence beta-3-adrenoceptor-mediated relaxation of the female rat detrusor muscle. *Urology,* **59,** 621–625.

MATSUI, M., GRIFFIN, M.T., SHEHNAZ, D., TAKETO, M.M. & EHLERT, F.J. (2003). Increased relaxant action of forskolin and isoproterenol against muscarinic agonist-induced contractions in smooth muscle from M_2 receptor knockout mice. *J. Pharmacol. Exp. Ther.,* **305,** 106–113.

MCCONNELL, J.D., ROEHRBORN, C.G., BAUTISTA, O., ANDRIOLE, G.L., DIXON, C.M., KUSEK, J.W., LEPOR, H., MCVARY, K.T., NYBERG, L.M., CLARKE, H.S., CRAWFORD, E.D., DIOKNO, A.C., FOLEY, J.P., FOSTER, H.E., JACOBS, S.C., KAPLAN, S.A., KREDER, K.J., LIEBER, M.M., LUCIA, M.S., MILLER, G.J., MENON, M., MILAM, D.F., RAMSDELL, J.W., SCHENKMAN, N.S., SLAWIN, K.M. & SMITH, J.A. (2003). The long-term effect of doxazosin, finasteride, and combination therapy on the clinical progression of benign prostatic hyperplasia. *New Engl. J. Med.,* **349,** 2387–2398.

MICHEL, M.C. (2002). Potential role of α_1-adrenoceptor subtypes in the aetiology of LUTS. *Eur. Urol. Suppl.,* **1,** 5–13.

MICHEL, M.C. & INSEL, P.A. (1994). Comparison of cloned and pharmacologically defined rat tissue α_1-adrenoceptor subtypes. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **350,** 136–142.

MICHEL, M.C., FLANNERY, M.T. & NARAYAN, P. (2001). Worldwide experience with alfuzosin and tamsulosin. *Urology,* **58,** 508–516.

MICHEL, M.C., GRÜBBEL, B., TAGUCHI, K., VERFÜRTH, F., OTTO, T. & KRÖPFL, D. (1996). Drugs for treatment of benign prostatic hyperplasia: affinity comparison at cloned α_1-adrenoceptor subtypes and in human prostate. *J. Auton. Pharmacol.,* **16,** 21–28.

MICHEL, M.C., KERKER, J., BRANCHEK, T.A. & FORRAY, C. (1993). Selective irreversible binding of chloroethylclonidine at α_1- and α_2-adrenoceptor subtypes. *Mol. Pharmacol.,* **44,** 1165–1170.

MICHEL, M.C., KORSTANJE, C., KRAUWINKEL, W., SHEAR, M., DAVIES, J. & QUARTEL, A. (2005a). Cardiovascular safety of the oral controlled absorption system (OCAS) formulation of tamsulosin compared to the modified release (MR) formulation. *Eur. Urol. Suppl.,* **4,** 53–60.

MICHEL, M.C., KORSTANJE, C., KRAUWINKEL, W., SHEAR, M., DAVIES, J. & QUARTEL, A. (2005b). Comparison of vascular α_1-adrenoceptor antagonism of tamsulosin oral controlled absorption system (OCAS) and modified release (MR) formulations. *Eur. Urol. Suppl.,* **4,** 45–52.

MICHEL, M.C., MEHLBURGER, L., SCHUMACHER, H., BRESSEL, H.-U. & GOEPEL, M. (2000a). Effect of diabetes on lower urinary tract symptoms in patients with benign prostatic hyperplasia. *J. Urol.,* **163,** 1725–1729.

MICHEL, M.C., OELKE, M. & PETERS, S.L.M. (2005c). The neuro–urological connection. *Eur. Urol. Suppl.,* **4,** 18–28.

MICHEL, M.C., SCHÄFERS, R.F. & GOEPEL, M. (2000b). α-Blockers and lower urinary tract function: more than smooth muscle relaxation? *BJU Int.,* **86** (Suppl. 2), 23–30.

MILSOM, I., ABRAMS, P., CARDOZO, L., ROBERTS, R.G., THÜROFF, J.W. & WEIN, A.J. (2001). How widespread are the symptoms of an overactive bladder and how are they managed? A population-based prevalence study. *BJU Int.,* **87,** 760–766.

MONNERON, M.-C., GILLBERG, P.G., OHMAN, B. & ALBERTS, P. (2000). *In vitro* α-adrenoceptor autoradiography of the urethra and urinary bladder of the female pig, cat, guinea-pig and rat. *Scand. J. Urol. Nephrol.,* **34,** 233–238.

MORITA, T. & KONDO, S. (1992a). Comparison of selective alpha-1 blockade for alpha-receptors in human hypertrophied prostatic adenomas. *Japan. J. Urol.,* **3,** 334–337.

MORITA, T. & KONDO, S. (1992b). Quantitative analysis of alpha-adrenoceptors in human prostatic capsule, adenoma and urethra – a comparison between normal and hypertrophied prostate. *Nippon Hinyokika Gakkai Zasshi,* **83,** 328–333.

MORITA, T., IIZUKA, H., IWATA, T. & KONDO, S. (2000). Function and distribution of β_3-adrenoceptors in rat, rabbit and human urinary bladder and external urethral sphincter. *J. Smooth Muscle Res.,* **36,** 21–32.

MORITA, T., MASUDA, H., TOSAKA, A., ISHIZAKA, K., TSUHII, T. & KONDO, S. (1998). Sex differences in function and distribution of β-adrenoceptors in rabbit urinary bladder. *J. Urol.,* **159,** 555–558.

MORIYAMA, N., KURIMOTO, S., HORIE, S., NASU, K., TANAKA, T., YANO, K., HIRANO, H., TSUJIMOTO, G. & KAWABE, K. (1996). Detection of α_1-adrenoceptor subtypes in human hypertrophied prostate by *in situ* hybridization. *Histochem. J.,* **28,** 283–288.

MORIYAMA, N., YAMAGUCHI, T., TAKEUCHI, T., SAKAMOTO, E., UEKI, T., TSUJIMOTO, G. & KAWABE, K. (1998). Semiquantitative evaluation of α_{1A}-adrenoceptor subtype mRNA in human hypertrophied and non-hypertrophied prostates: regional comparison. *Life Sci.,* **64,** 201–210.

MOTTET, N., BREESSOLE, F., DELMAS, V., ROBERT, M. & COSTA, P. (2003). Prostatic tissue distribution of alfuzosin in patients with benign prostatic hyperplasia following repeated oral administration. *Eur. Urol.,* **44,** 101–105.

MURAMATSU, I., OHMURA, T., KIGOSHI, S., HASHIMOTO, S. & OSHITA, M. (1990). Pharmacological subclassification of α_1-adrenoceptors in vascular smooth muscle. *Br. J. Pharmacol.,* **99,** 197–201.

MUSSELMAN, D.M., FORD, A.P.D.W., GENNEVOIS, D.J., HARBISON, M.L., LAURENT, A.L., MOKATRIN, A.S., STOLTZ, R.R. & BLUE, D.R. (2004). A randomized crossover study to evaluate Ro 115–1240, a selective $\alpha_{1A/L}$-adrenoceptor partial agonist in women with stress urinary incontinence. *BJU Int.,* **93,** 78–83.

MUZZIN, P., BOSS, O., MATHIS, N., REVELLI, J.-P., GIACOBINO, J.-P., WILLCOCKS, K., BADMAN, G.F., CANTELLO, B.C.C., HINDLEY, R.M. & CAWTHORNE, M.A. (1994). Characterization of a new, highly specific, β_3-adrenergic receptor radioligand, [^{3}H]SB 206606. *Mol. Pharmacol.,* **46,** 357–363.

NAGMANI, R., PASCO, D.S., SALAS, R.D. & FELLER, D.R. (2003). Evaluation of β-adrenergic receptor subtypes in the human prostate cancer cell line-LNCaP. *Biochem. Pharmacol.,* **65,** 1489–1494.

NAKAHARA, T., KUBOTA, Y., MITANI, A., MARUKO, T., SAKAMOTO, K. & ISHII, K. (2003). Protease-activated receptor-2-mediated contraction in the rat urinary bladder: the role of urinary bladder mucosa. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **367,** 211–213.

NAKAHARA, T., KUBOTA, Y., SAITO, M., SAKAMOTO, K. & ISHII, K. (2004). Protease-activated receptor-2-mediated contraction of urinary bladder is enhanced in cyclophosphamide-treated rats. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **369,** 212–219.

NAKAHIRA, Y., HASHITANI, H., FUKUTA, H., SASAKI, S., KOHRI, K. & SUZUKI, H. (2001). Effects of isoproterenol on spontaneous excitations in detrusor muscle cells of the guinea pig. *J. Urol.,* **166,** 335–340.

NAKAMURA, T., YOSHIMURA, M., SHINNICK-GALLAGHER, P., GALLAGHER, J.P. & AKASU, T. (1984). α_2 and α_1-Adrenoceptors mediate opposite actions on parasympathetic neurons. *Brain Res.,* **323,** 349–353.

NASU, K., MORIYAMA, N., FUKASAWA, R., TSUJIMOTO, G., TANAKA, T., YANO, J. & KAWABE, K. (1998). Quantification and distribution of α_1-adrenoceptor subtype mRNAs in human proximal urethra. *Br. J. Pharmacol.,* **123,** 1289–1293.

NASU, K., MORIYAMA, N., KAWABE, K., TSUJIMOTO, G., MURAI, M., TANAKA, T. & YANO, J. (1996). Quantification and distribution of α1-adrenoceptor subtype mRNAs in human prostate: comparison of benign hypertrophied tissue and non-hypertrophied tissue. *Br. J. Pharmacol.,* **119,** 797–803.

NERGARDH, A., BOREUS, L.O. & NAGLO, A.S. (1977). Characterization of the adrenergic beta-receptor in the urinary bladder of man and cat. *Acta Pharmacol. Toxicol.,* **40,** 14–21.

NISHIMOTO, T., LATIFPOUR, J., WHEELER, M.A., YOSHIDA, M. & WEISS, R.M. (1995). Age-dependent alterations in β-adrenergic responsiveness of rat detrusor smooth muscle. *J. Urol.,* **153,** 1701–1705.

NOBLE, A.J., CHESS-WILLIAMS, R., COULDWELL, C., FURUKAWA, K., UCHYIUMA, T., KORSTANJE, C. & CHAPPLE, C.R. (1997). The effects of tamsulosin, a high affinity antagonist at functional α_{1A}- and α_{1D}-adrenoceptor subtypes. *Br. J. Pharmacol.,* **120,** 231–238.

NOMIYA, M. & YAMAGUCHI, O. (2003). A quantitative analysis of mRNA expression of α1 and β-adrenoceptor subtypes and their functional roles in human normal and obstructed bladders. *J. Urol.,* **170,** 649–653.

NORLEN, L., SUNDIN, T. & WAAGSTEIN, F. (1978). Effect of β-adrenoceptor stimulation on the human bladder *in vivo*. *Urol. Int.,* **33,** 355–358.

NORMANDIN, D.E. & LODGE, N.J. (1996). Pharmacological characterization of the isolated canine prostate. *J. Urol.,* **155,** 1758–1761.

OBIKA, K., SHIBATA, A., HORIE, K., FOGLAR, R., KIMURA, K. & TSUJIMOTO, G. (1995). NS-49, a novel α_{1a}-adrenoceptor-selective agonist characterization using recombinant human α_1-adrenoceptors. *Eur. J. Pharmacol.,* **291,** 327–334.

OHMI, K., SHINOURA, H., NAKAYAMA, Y., GODA, N. & TSUJIMOTO, G. (1999). Characterization of α_1-adrenoceptors expressed in a novel vascular smooth muscle cell line cloned from p53 knockout mice, P53LMAC01 (AC01) cells. *Br. J. Pharmacol.,* **127,** 756–762.

OHMURA, T., OSHITA, M., KIGOSHI, S. & MURAMATSU, I. (1992). Identification of α_1-adrenoceptor subtypes in the rat vas deferens: binding and functional studies. *Br. J. Pharmacol.,* **107,** 697–704.

OHMURA, T., SAKAMOTO, S., HAYASHI, H., KIGOSHI, S. & MURAMATSU, I. (1993). Identification of α_1-adrenoceptor subtypes in the dog prostate. *Urol. Res.,* **21,** 211–215.

OSHITA, M., HIRAOKA, Y. & WATANABE, Y. (1997). Characterization of β-adrenoceptors in urinary bladder: comparison between rat and rabbit. *Br. J. Pharmacol.,* **122,** 1720–1724.

PERÄLÄ, M., HIRVONEN, H., KALIMO, H., ALA-UOTILA, S., REGAN, J.W., AKERMAN, K.E.O. & SCHEININ, M. (1992). Differential expression of two α_2-adrenergic receptor subtype mRNAs in human tissues. *Mol. Brain Res.,* **16,** 57–63.

PETERS, S.L.M. & MICHEL, M.C. (2003). cAMP-independent relaxation of smooth muscle cells *via* G_s-coupled receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **368,** 329–330.

PETKOV, G.V. & NELSON, M.T. (2005). Differential regulation of Ca^{2+}-activated K^+ channels by β-adrenoceptors in guinea pig urinary bladder smooth muscle. *Am. J. Physiol.,* **288,** C1255–C1263.

POYET, P., GAGNE, B. & LABRIE, F. (1986a). Characteristics of the β-adrenergic stimulation of adenylate cyclase activity in rat ventral prostate and its modulation by androgens. *Prostate,* **9,** 237–245.

POYET, P., GAGNE, B., LAVOIE, M. & LABRIE, F. (1986b). Characteristics of the β-adrenergic receptor in the rat ventral prostate using [^{125}I]cyanopindolol. *Mol. Cell. Endocrinol.,* **48,** 59–67.

PRICE, D.T., SCHWINN, D.A., LOMASNEY, J.W., ALLEN, L.F., CARON, M.G. & LEFKOWITZ, R.J. (1993). Identification, quantification, and localization of mRNA for three distinct alpha$_1$ adrenergic receptor subtypes in human prostate. *J. Urol.,* **150,** 546–551.

PULITO, V.L., LI, X., VARGA, S.S., MULCAHY, L.S., CLARK, K.S., HALBERT, S.A., REITZ, A.B., MURRAY, W.V. & JOLLIFFE, L.K. (2000). An investigation of the uroselective properties of four novel α_{1a}-adrenergic receptor subtype-selective antagonists. *J. Pharmacol. Exp. Ther.,* **294,** 224–229.

PURVIS, K., RUI, H., GORDELADZE, J.O. & ATTRAMADAL, H. (1986). Hormonal activation of the adenylyl cyclases of the rat and human prostate gland. *Prostate,* **8,** 11–24.

RAMAGE, A.G. & WYLLIE, M.G. (1995). A comparison of the effects of doxazosin and terazosin on the spontaneous sympathetic drive to the bladder and related organs in anaesthetized cats. *Eur. J. Pharmacol.,* **294,** 645–650.

RAMASAMY, S., HODGSON, W.C. & VENTURA, S. (2002). Protein kinase C and the sub-sensitivity and sub-reactivity of the diabetic rat prostate gland to noradrenaline. *Eur. J. Pharmacol.,* **434,** 151–161.

ROEHRBORN, C., VAN KERREBROECK, P. & NORDLING, J. (2003). Safety and efficacy of alfuzosin 10 mg once-daily in the treatment of lower urinary tract symptoms and clinical benign prostatic hyperplasia: a pooled analysis of three double-blind, placebo-controlled studies. *BJU Int.,* **92,** 257–261.

ROEHRBORN, C.G. & SCHWINN, D.A. (2004). α_1-Adrenergic receptors and their inhibitors in lower urinary tract symptoms and benign prostatic hyperplasia. *J. Urol.,* **171,** 1029–1035.

ROKOSH, D.G., BAILEY, B.A., STEWART, A.F.R., KARNS, L.R., LONG, C.S. & SIMPSON, P.C. (1994). Distribution of α1C-adrenergic receptor mRNA in adult rat tissues by RNase protection assay and comparison with α1B and α1D. *Biochem. Biophys. Res. Commun.,* **200,** 1177–1184.

ROMIC, I., KISS, T., KISBENEDEK, L., KONDAS, J., TORZSOK, F., MILAK, M., AVIS, M., KRAUWINKEL, W., SWART, P. & KORSTANJE, C. (2003). Tamsulosin drug ratio in prostate *versus* free fraction in plasma supports pharmacokinetic (PK) contribution to its uroselectivity. *J. Urol.,* **169** (Suppl), 288.

RUDNER, X.L., BERKOWITZ, B.A., BOOTH, J.V., FUNK, B.L., COZART, K.L., D'AMICO, E.B., EL-MOALEM, H., PAGE, S.O., RICHARDSON, C.D., WINTERS, B., MARUCCI, L. & SCHWINN, D.A. (1999). Subtype specific regulation of human vascular α_1-adrenergic receptors by vessel bed and age. *Circulation,* **100,** 2336–2343.

SANTICIOLI, P., MAGGI, C.A. & MELI, A. (1983). The effects of clonidine on electrically-induced contractions of rat detrusor strips *in vitro*. *J. Auton. Pharmacol.,* **3,** 161–166.

SATO, S., OHTAKE, A., MATSUSHIMA, H., SAITOH, C., USUDA, S. & MIYATA, K. (2001). Pharmacological effect of tamsulosin in relation to dog plasma and tissue concentrations: prostatic and urethral retention possibly contributes to uroselectivity of tamsulosin. *J. Pharmacol. Exp. Ther.,* **296,** 697–703.

SCHNEIDER, T., HEIN, P., MICHEL-REHER, M. & MICHEL, M.C. (2005). Effects of ageing on muscarinic receptor subtypes and function in rat urinary bladder. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **372,** 71–78.

SCOFIELD, M.A., LIU, F., ABEL, P.W. & JEFFRIES, W.B. (1995). Quantification of steady state expression of mRNA for alpha-1 adrenergic receptor subtypes using reverse transcription and a competitive polymerase chain reaction. *J. Pharmacol. Exp. Ther.,* **275,** 1035–1042.

SEGUCHI, H., NISHIMURA, J., ZHOU, Y., NIIRO, N., KUMAZAWA, J. & KANAIDE, H. (1998). Expression of β_3-adrenoceptors in rat detrusor muscle. *J. Urol.,* **159,** 2197–2201.

SHAPIRO, E. & LEPOR, H. (1986). α_2 adrenergic receptors in hyperplastic human prostate: identification and characterization using [^{3}H]rauwolscine. *J. Urol.,* **135,** 1038–1042.

SHAPIRO, E., BECICH, M.J., HARTANTO, V. & LEPOR, H. (1992). The relative proportion of stromal and epithelial hyperplasia is related to the development of symptomatic benign prostatic hyperplasia. *J. Urol.,* **147,** 1293–1297.

SHAPIRO, E., TSITLIJK, J.E. & LEPOR, H. (1987). α_2 adrenergic receptors in canine prostate: biochemical and functional correlations. *J. Urol.,* **137,** 565–570.

SHIMA, S., KAWASHIMA, Y., HIRAI, M. & ASAKURA, M. (1980). Effect of adrenergic stimulation on adenylate cyclase activity in rat prostate. *Biochim. Biophys. Acta,* **628,** 255–262.

SIGALA, S., PERONI, A., MIRABELLA, G., FORNARI, S., PALAZZOLO, F., PEZZOTTI, G., SIMEONE, C., COSCIANI CUNICO, S. & SPANO, P.F. (2004). Alpha1 adrenoceptor subtypes in human urinary bladder: sex and regional comparison. *Life Sci.,* **76,** 417–427.

SLATER, M., BARDEN, J.A. & MURPHY, C.R. (2000). Tyrosine kinase A, autonomic and transmitter receptors, but not innervation, are upregulated in the aging rat prostate. *Acta Histochem.,* **102,** 427–438.

SMITH, L.M., KAJIOKA, S., BRADING, A.F. & NAKAYAMA, S. (1999). Effects of phosphorylation-related drugs on slow Ca^{2+} tail current in guinea-pig detrusor cells. *Eur. J. Pharmacol.,* **370,** 187–193.

SOLANO, R.M., CARMENA, M.J., GUIJARRO, L.G. & PRIETO, J.C. (1994). Neuropeptide Y inhibits vasoactive intestinal peptide-stimulated adenylyl cyclase in rat ventral prostate. *Neuropeptides,* **27,** 31–37.

SOMERS, W.J., FELSEN, D., CHOU, T.C., MARION, D.N., CHERNESKY, C.E. & VAUGHAN JR, E.D. (1989). An *in vivo* evaluation of alpha adrenergic receptors in canine prostate. *J. Urol.,* **141,** 1230–1233.

SOMOGYI, G.T., TANOWITZ, M. & DE GROAT, W.C. (1995). Prejunctional facilitatory α_1-adrenoceptors in the rat urinary bladder. *Br. J. Pharmacol.,* **114,** 1710–1716.

SPRINGER, J.P., KROPP, B.P. & THOR, K.B. (1994). Facilitatory and inhibitory effects of selective norepinephrine reuptake inhibitors on hypogastric nerve-evoked urethral contractions in the cat: a prominent role of urethral β-adrenergic receptors. *J. Urol.,* **152,** 515–519.

STEIDLE, C.P., COHEN, M.L., HOOVER, D.M. & NEUBAUER, B.L. (1989). Comparative contractile responses among ventral, dorsal, and lateral lobes of the rat prostate. *Prostate,* **15,** 53–63.

SUGAYA, K., NISHIJIMA, S., MIYAZATO, M., ASHITOMI, K., HATANO, T. & OGAWA, Y. (2002). Effects of intrathecal injection of tamsulosin and naftopidil, alpha-1A and -1D adrenergic receptor antagonists, on bladder activity in rats. *Neurosci. Lett.,* **328,** 74–76.

SUZUKI, F., MIYAMOTO, S., TAKITA, M., OSHITA, M., WATANABE, Y., KAKIZUKA, A., NARUMIYA, S., TANIGUCHI, T. & MURAMATSU, I. (1997). Cloning, functional expression and tissue distribution of rabbit α_{1d}-adrenoceptor. *Biochim. Biophys. Acta,* **1323,** 6–11.

SWIERZEWSKI, S.J.I., GORMLEY, E.A., BELVILLE, W.D., SWEETSER, P.M., WAN, J. & MCGUIRE, E.J. (1994). The effect of terazosin on bladder function in the spinal cord injured patient. *J. Urol.,* **151,** 951–954.

SZELL, E.A., YAMAMOTO, T., DE GROAT, W.C. & SOMOGYI, G.T. (2000). Smooth muscle and parasympathetic nerve terminals in the rat urinary bladder have different subtypes of α_1 adrenoceptors. *Br. J. Pharmacol.,* **130,** 1685–1691.

TAGUCHI, K., SAITOH, M., SATO, S., ASANO, M. & MICHEL, M.C. (1997). Effects of tamsulosin metabolites at alpha-1 adrenoceptor subtypes. *J. Pharmacol. Exp. Ther.,* **280,** 1–5.

TAHMATZOPOULOS, A. & KYPRIANOU, N. (2004). Apoptotic impact of α_1-blockers on prostate cancer growth: a myth or an inviting reality? *Prostate,* **59,** 91–100.

TAKAHASHI, S., MORIYAMA, N., YAMAZAKI, R. & KAWABE, K. (1996). Urodynamic analysis of age-related changes of α_1-adrenoceptor responsiveness in female beagle dogs. *J. Urol.,* **156,** 1485–1488.

TAKAHASHI, W., AFIATPOUR, P., FOSTER JR, H.E., IKEDA, K., WADA, Y., WEIS, R.M. & LATIFPOUR, J. (2002). The effect of castration on endothelins, their receptors and endothelin converting enzyme in rat prostate. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **366,** 166–176.

TAKEDA, H., IGAWA, Y., KOMATSU, Y., YAMAZAKI, Y., AKAHANE, M., NISHIZAWA, O. & AJISAWA, Y. (2000a). Characterization of β-adrenoceptor subtypes in the ferret urinary bladder *in vitro* and *in vivo*. *Eur. J. Pharmacol.,* **403,** 147–155.

TAKEDA, H., MATSUZAWA, A., IGAWA, Y., YAMAZAKI, Y., KAIDOH, K., AKAHANE, S., KOJIMA, M., MIYATA, H., AKAHANE, M. & NISHIZAWA, O. (2003). Functional characterization of β-adrenoceptor subtypes in the canine and rat lower urinary tract. *J. Urol.,* **170,** 654–658.

TAKEDA, H., YAMAZAKI, Y., AKAHANE, M., AKAHANE, S., MIYATA, H., IGAWA, Y. & NISHIZAWA, O. (2002a). Characterization of β-adrenoceptor subtype in bladder smooth muscle of cynomolgus monkey. *Jpn. J. Pharmacol.,* **88,** 108–113.

TAKEDA, H., YAMAZAKI, Y., AKAHANE, M., IGAWA, Y., AJISAWA, Y. & NISHIZAWA, O. (2000b). Role of β_3-adrenoceptor in urine storage in the rat: comparison between the selective β_3-adrenoceptor agonist, CL316, 243, and various smooth muscle relaxants. *J. Pharmacol. Exp. Ther.,* **293,** 939–945.

TAKEDA, H., YAMAZAKI, Y., IGAWA, Y., KAIDOH, K., AKAHANE, S., MIYATA, H., NISHIZAWA, O., AKAHANE, M. & ANDERSSON, K.-E. (2002b). Effects of β_3-adrenoceptor stimulation on prostaglandin E_2-induced bladder hyperreactivity and on the cardiovascular system in conscious rats. *Neurourol. Urodyn.,* **21,** 558–565.

TAKEDA, M., OBARA, K., MIZUSAWA, T., TOMITA, Y., ARAI, K., TSUTSUI, T., HATANO, A., TAKAHASHI, K. & NOMURA, S. (1999). Evidence for β_3-adrenoceptor subtypes in relaxation of the human urinary bladder detrusor: analysis by molecular biological and pharmacologocial methods. *J. Pharmacol. Exp. Ther.,* **288,** 1367–1373.

TANAKA, Y., YAMASHITA, Y., YAMAKI, F., HORINOUCHI, T., SHIGENOBU, K. & KOIKE, K. (2003). Evidence for a significant role of a G_s-triggered mechanism unrelated to the activation of adenylyl cyclase in the cyclic AMP-independent relaxant response of guinea-pig tracheal smooth muscle. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **368,** 437–441.

TENG, C.-M., GUH, J.-H. & KO, F.-N. (1994). Functional identification of α_1-adrenoceptor subtypes in human prostate: comparison with those in rat vas deferens and spleen. *Eur. J. Pharmacol.,* **265,** 61–66.

TESTA, R., GUARNERI, L., ANGELICO, P., POGGESI, E., TADDEI, C., SIRONI, G., COLOMBO, D., SULPIZIO, A.C., NASELSKY, D.P., HIEBLE, J.P. & LEONARDI, A. (1997). Pharmacological characterization of the uroselective alpha-1 antagonist Rec 15/2739 (SB 216469): role of the alpha-1L adrenoceptor in tissue selectivity, part II. *J. Pharmacol. Exp. Ther.,* **281,** 1284–1293.

TESTA, R., GUARNERI, L., IBBA, M., STRADA, G., POGGESI, E., TADDEI, C., SIMONAZZI, I. & LEONARDI, A. (1993). Characterization of α_1-adrenoceptor subtypes in prostate and prostatic urethra of rat, rabbit, dog and man. *Eur. J. Pharmacol.,* **249,** 307–315.

TESTA, R., GUARNERI, L., TADDEI, C., POGGESI, E., ANGELICO, P., SARTANI, A., LEONARDI, A., GOFRIT, O.N., MERETYK, S. & CAINE, M. (1996). Functional antagonistic activity of Rec 15/2739, a novel alpha-1 antagonist selective for the lower urinary tract, on noradrenaline-induced contraction of human prostate and mesenteric artery. *J. Pharmacol. Exp. Ther.,* **277,** 1237–1246.

THIND, P., LOSE, G., COLSTRUP, H. & ANDERSSON, K.-E. (1992). The effects of α-adrenoceptor stimulation and blockade on the static urethral sphincter function in healthy females. *Scand. J. Urol. Nephrol.,* **26,** 219–225.

THIND, P., LOSE, G., COLSTRUP, H. & ANDERSSON, K.-E. (1993a). The effect of pharmacological stimulation and blockade of autonomic receptors on the urethral pressure and power generation during coughing and squeezing of the pelvic floor in healthy females. *Scand. J. Urol. Nephrol.,* **27,** 519–525.

THIND, P., LOSE, G., COLSTRUP, H. & ANDERSSON, K.-E. (1993b). The influence of β-adrenoceptor and muscarinic receptor agonists and antagonists on the static urethral closure function in healthy females. *Scand. J. Urol. Nephrol.,* **27,** 31–38.

TSENG-CRANK, J., KOST, T., GOETZ, A., HAZUM, S., ROBERSON, K.M., HAIZLIP, J., GODINOT, N., ROBERTSON, C.N. & SAUSSY, D. (1995). The α_{1C}-adrenoceptor in human prostate: cloning, functional expression, and localization to specific prostatic cell types. *Br. J. Pharmacol.,* **115,** 1475–1485.

TSUJII, T., AZUMA, H., YAMAGUCHI, T. & OSHIMA, H. (1992). A possible role of decreased relaxation mediated by β-adrenoceptors in bladder outlet obstruction by benign prostatic hypertrophy. *Br. J. Pharmacol.,* **107,** 803–807.

TSUJIMOTO, G., TIMMINS, P.V. & HOFFMAN, B.B. (1986). Alpha adrenergic receptors in the rabbit bladder base smooth muscle: alpha-1 adrenergic receptors mediate contractile responses. *J. Pharmacol. Exp. Ther.,* **236,** 384–389.

TSURUSAKI, M., YOSHIDA, M., AKASU, T. & NAGATSU, I. (1990). α_2-Adrenoceptors mediate the inhibition of cholinergic transmission in parasympathetic ganglia of the rabbit urinary bladder. *Synapse,* **5,** 233–240.

TUCCI, P., BARTOCCI, C. & BOLLE, P. (2002). Cyclo-oxygenase- and capsaicin-sensitive afferent fibres affect beta-adrenoceptor-evoked response in the rat urinary bladder. *Pharmacology,* **64,** 57–62.

TUGAY, M., YILDIZ, F., UTKAN, T., GACAR, N., ULAK, G. & ERDEN, F. (2003). Age-related smooth muscle reactivity changes in the rat bladder: an *in vitro* study. *Pharmacol. Res.,* **48,** 329–334.

UCHIDA, H., SHISHIDO, K., NOMIYA, M. & YAMAGUCHI, O. (2005). Involvement of cyclic AMP-dependent and -independent mechanisms in the relaxation of rat detrusor muscle *via* β-adrenoceptors. *Eur. J. Pharmacol.,* **518,** 195–202.

UEDA, S., SATAKE, N. & SHIBATA, S. (1984). α_1- and α_2-adrenoceptors in the smooth muscle of isolated rabbity urinary bladder and urethra. *Eur. J. Pharmacol.,* **103,** 249–254.

VAN DER GRAAF, P.H., DEPLANNE, V., DUQUENNE, C. & ANGEL, I. (1997). Analysis of α_1-adrenoceptors in rabbit lower urinary tract and mesenteric artery. *Eur. J. Pharmacol.,* **327,** 25–32.

VAN DER WERF, B.A. & CREED, K.E. (2002). Mechanical properties and innervation of the smooth muscle layers of the urethra of greyhounds. *BJU Int.,* **90,** 588–595.

WAGHE, M., WESTWOOD, R., NUNN, G., KALINOWSKI, A. & ALDRIDGE, A. (1999). Urinary tract toxicity in rats following administration of beta 3-adrenoceptor agonists. *Toxicol. Pathol.,* **27,** 165–170.

WALDEN, P.D., DURKIN, M.M., LEPOR, H., WETZEL, J.M., GLUCHOWSKI, C. & GUSTAFSON, E.L. (1997). Localization of mRNA and receptor binding sites for the α_{1A}-adrenoceptor subtype in the rat, monkey and human urinary bladder and prostate. *J. Urol.,* **157,** 1032–1038.

WALDEN, P.D., GLOBINA, Y. & NIEDER, A. (2004). Induction of anoikis by doxazosin in prostate cancer cells is associated with activation of caspase-3 and a reduction of focal adhesion kinase. *Urol. Res.,* **32,** 261–265.

WEINBERG, D.H., TRIVEDI, P., TAN, C.P., MITRA, S., PERKINS-BARROW, A., BORKOWSKI, D., STRADER, C.D. & BAYNE, M. (1994). Cloning, expression and characterization of human α adrenergic receptors α_{1A}, α_{1B} and α_{1C}. *Biochem. Biophys. Res. Commun.,* **201,** 1296–1304.

WILLIAMS, T.J., BLUE, D.R., DANIELS, D.V., DAVIS, B., ELWORTHY, T., GEBER, J.R., KAVA, M.S., MORGANS, D., PADILLA, F., TASSA, S., VIMONT, R.L., CHAPPLE, C.R., CHESS-WILLIAMS, R., EGLEN, R.M., CLARKE, D.E. & FORD, A.P.D.W. (1999). *In vitro* α_1-adrenoceptor pharmacology of Ro 70.0004 and RS-100329, novel α_{1A}-adrenoceptor selective antagonists. *Br. J. Pharmacol.,* **127,** 252–258.

WITJES, W.P.J., ROSIER, P.F.W.M., CARIS, C.T.M., DEBRUYNE, F.M.J. & DE LA ROSETTE, J.J.M.C.H. (1997). Urodynamic and clinical effects of terazosin therapy in symptomatic patients with and without bladder outflow obstruction: a stratified analysis. *Urology,* **49,** 197–206.

WITTE, D.G., BRUNE, M.E., KATWALA, S.P., MILICIC, I., KERWIN JR, J.F. & HANCOCK, A.A. (1997). Relationships between pharmacokinetics and blockade of agonist-induced prostatic intraurethral pressure and mean arterial pressure in the conscious dog after single and repeated daily oral administration of terazosin. *J. Pharmacol. Exp. Ther.,* **282,** 891–898.

WITTE, D.G., BRUNE, M.E., KATWALA, S.P., MILICIC, I., STOLARIK, D., HUI, Y.-H., MARSH, K.C., KERWIN JR, J.F., MEYER, M.D. & HANCOCK, A.A. (2002). Modeling of relationships between pharmacokinetics and blockade of agonist-induced elevation of intraurethral pressure and mean arterial pressure in conscious dogs treated with α_1-adrenoceptor antagonists. *J. Pharmacol. Exp. Ther.,* **300,** 495–504.

WOODS, M., CARSON, N., NORTON, N.W., SHELDON, J.H. & ARGENTIERI, T.M. (2001). Efficacy of the β3-adrenergic receptor agonist CL-316243 on experimental bladder hyperreflexia and detrusor instability in the rat. *J. Urol.,* **166,** 1142–1147.

YAMADA, S., SUZUKI, M., KATO, Y., KIMURA, R., MORI, R., MATSUMOTO, K., MARUYAMA, M. & KAWABE, K. (1992). Binding characteristics of naftopidil and α_1-adrenoceptor antagonists to prostatic α-adrenoceptors in benign prostatic hypertrophy. *Life Sci.,* **50,** 127–135.

YAMAGISHI, R., AKIYAMA, K., NAKAMURA, S., HORA, M., MASUDA, N., MATSUZAWA, A., MURATA, S., UJIIE, A., KURASHINA, Y., IIZUKA, K. & KITAZAWA, M. (1996). Effect of KMD-3213, an α_{1a}-adrenoceptor-selective antagonist, on the contractions of rabbit prostate and rabbit and rat aorta. *Eur. J. Pharmacol.,* **315,** 73–79.

YAMAMOTO, Y.L., MORI, A. & KOIKE, K. (1998). Adrenoceptor in the detrusor of guinea pig bladder. *J. Smooth Muscle Res.,* **34,** 233–242.

YAMANISHI, T., CHAPPLE, C.R., YASUDA, K., YOSHIDA, K. & CHESS-WILLIAMS, R. (2002b). The role of β_3-adrenoceptors in mediating relaxation of porcine detrusor muscle. *Br. J. Pharmacol.,* **135,** 129–134.

YAMANISHI, T., CHAPPLE, C.R., YASUDA, K., YOSHIDA, K. & CHESS-WILLIAMS, R. (2002a). The role of M_2 muscarinic receptor subtypes mediating contraction of the circular and longitudinal smooth muscle of the pig proximal urethra. *J. Urol.,* **168,** 308–314.

YAMANISHI, T., CHAPPLE, C.R., YASUDA, K., YOSHIDA, K. & CHESS-WILLIAMS, R. (2003a). Role of β-adrenoceptor subtypes in mediating relaxation of the pig bladder trigonal muscle *in vitro*. *Neurourol. Urodyn.,* **22,** 338–342.

YAMANISHI, T., CHAPPLE, C.R., YASUDA, K., YOSHIDA, K. & CHESS-WILLIAMS, R. (2002c). Identification of β-adrenoceptor subtypes in lower urinary tract of the female pig. *J. Urol.,* **168,** 2706–2710.

YAMANISHI, T., CHAPPLE, C.R., YASUDA, K., YOSHIDA, K. & CHESS-WILLIAMS, R. (2003b). The functional role of β-adrenoceptor subtypes in mediating relaxation of pig urethral smooth muscle. *J. Urol.,* **170,** 2508–2511.

YAMAZAKI, Y., TAKEDA, H., AKAHANE, M., IGAWA, Y., NISHIZAWA, O. & AJISAWA, Y. (1998). Species differences in the distribution of β-adrenoceptor subtypes in bladder smooth muscle. *Br. J. Pharmacol.,* **124,** 593–599.

YANG, M., BÜSCHER, R., TAGUCHI, K., GRÜBBEL, B., INSEL, P.A. & MICHEL, M.C. (1998a). Protein kinase C does not mediate phenylephrine-induced down-regulation of Madin–Darby canine kidney cell alpha-1B adrenoceptors. *J. Pharmacol. Exp. Ther.,* **286,** 36–43.

YANG, M., REESE, J., COTECCHIA, S. & MICHEL, M.C. (1998b). Murine alpha$_1$-adrenoceptor subtypes. I. Radioligand binding studies. *J. Pharmacol. Exp. Ther.,* **286,** 841–847.

YANG, M., RUAN, J., VOLLER, M., SCHALKEN, J. & MICHEL, M.C. (1999). Differential regulation of human α_1-adrenoceptor subtypes. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **359,** 439–446.

YANG, M., VERFÜRTH, F., BÜSCHER, R. & MICHEL, M.C. (1997). Is α_{1D}-adrenoceptor protein detectable in rat tissues? *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **355,** 438–446.

YAZAWA, H. & HONDA, K. (1993). α_1-Adrenoceptor subtype in the rat prostate is preferentially the a_{1A} type. *Jpn. J. Pharmacol.,* **62,** 297–304.

YONO, M., FOSTER JR, H.E., TAKAHASHI, W., POURESMAIL, M. & LATIFPOUR, J. (2004). Doxazosin-induced up-regulation of α_{1A}-adrenoceptor mRNA in the rat lower urinary tract. *Can. J. Physiol. Pharmacol.,* **82,** 872–878.

YOSHIMURA, N., SASA, M., OHNO, Y., YOSHIDA, O. & TAKAORI, S. (1988). Contraction of urinary bladder by central norepinephrine originating in the locus coeruleus. *J. Urol.,* **139,** 423–427.

YOSHIYAMA, M. & DE GROAT, W.C. (2001). Role of spinal α_1-adrenoceptor subtypes in the bladder reflex in anesthetized rats. *Am. J. Physiol.,* **280,** R1414–R1419.

YU, S.-M., KO, F.-N., CHUEH, S.-C., CHEN, J., CHEN, S.-C., CHEN, C.-C. & TENG, C.-M. (1994). Effects of dicentrine, a novel α_1-adrenoceptor antagonist, on human hyperplastic prostates. *Eur. J. Pharmacol.,* **252,** 29–34.