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REVIEW

Phenotype pharmacology of lower urinary tract α_1-adrenoceptors

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α_1-Adrenoceptors are involved in numerous physiological functions, including micturition. However, the pharmacological profile of the α_1-adrenoceptor subtypes remains controversial. Here, we review the literature regarding α_1-adrenoceptors in the lower urinary tract from the standpoint of α_{1L} phenotype pharmacology. Among three α_1-adrenoceptor subtypes (α_{1A}, α_{1B} and α_{1D}), α_{1a}-adrenoceptor mRNA is the most abundantly transcribed in the prostate, urethra and bladder neck of many species, including humans. In prostate homogenates or membrane preparations, α_{1A}-adrenoceptors with high affinity for prazosin have been detected as radioligand binding sites. Functional α_1-adrenoceptors in the prostate, urethra and bladder neck have low affinity for prazosin, suggesting the presence of an atypical α_1-adrenoceptor phenotype (designated as α_{1L}). The α_{1L}-adrenoceptor occurs as a distinct binding entity from the α_{1A}-adrenoceptor in intact segments of variety of tissues including prostate. Both the α_{1L}- and α_{1A}-adrenoceptors are specifically absent from *Adra1A* (α_{1a}) gene-knockout mice. Transfection of α_{1a}-adrenoceptor cDNA predominantly expresses α_{1A}-phenotype in several cultured cell lines. However, in CHO cells, such transfection expresses α_{1L}- and α_{1A}-phenotypes. Under intact cell conditions, the α_{1L}-phenotype is predominant when co-expressed with the receptor interacting protein, CRELD1α. In summary, recent pharmacological studies reveal that two distinct α_1-adrenoceptor phenotypes (α_{1A} and α_{1L}) originate from a single *Adra1A* (α_{1a}-adrenoceptor) gene, but adrenergic contractions in the lower urinary tract are predominantly mediated via the α_{1L}-adrenoceptor. From the standpoint of phenotype pharmacology, it is likely that phenotype-based subtypes such as the α_{1L}-adrenoceptor will become new targets for drug development and pharmacotherapy.

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Abbreviations

BPH, benign prostatic hyperplasia; CRELD1α, cycteine-rich epidermal growth factor-like domain 1α; NS-49 (R)-(-)-3′-(2-amino-1-hydroxyethyl)-4′-fluoromethanesulphonanilide hydrochloride; Ro 115-1240 (Dabuzalgron, R-450), N-[6-chloro-3-(4,5-dihydro-1H-imidazol-2-ylmethoxy)-2-methylphenyl] methanesulphonamide; RS-17053, *N*-[2-(2-cyclopropylmethoxyphenoxy)ethyl]-5-chloro-α, α-dimethyl-1 *H*-indole-3-ethanamine hydrochloride; silodosin, 1-(3-hydroxypropyl)-5-[(2*R*)-({2-[2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethyl}amino) propyl] indoline-7-carboxamide; SUI, stress urinary incontinence; WB4101, 2(N[2,6-dimethyoxyphenoxyethyl])amino-methyl-1,4-benzodioxane

Introduction

The lower urinary tract is responsible for urine storage and voiding (Andersson and Wein, 2004). In mammalian species including humans, the bladder detrusor muscle contracts through a parasympathetic cholinergic mechanism during micturition (Abrams *et al.*, 2006; Wess *et al.*, 2007). Besides the importance of the cholinergic system as the major mechanism for bladder neck muscle tone control, the adrenergic systems play significant roles in the regulation of bladder neck tone (Fig. 1). During the storage phase, the urethra and outlet region of the bladder is contracted to

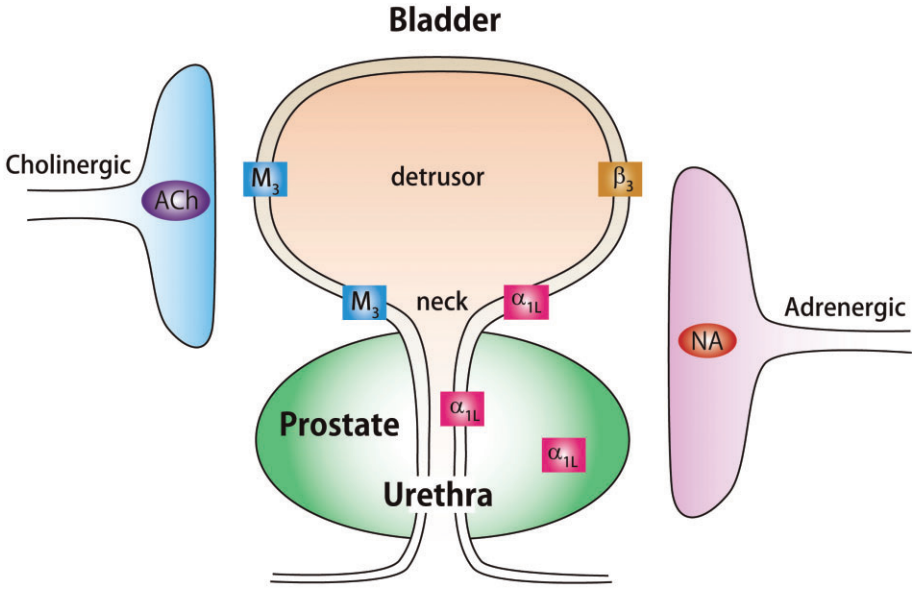

Figure 1

Autonomic innervation and main functional receptors involved in controlling smooth muscle tension in lower urinary tract tissues. NA, noradrenaline; α_{1L}, α_{1L}-adrenoceptor; β_3, β_3-adrenoceptor; M_3, M_3-muscarinic acetylcholine receptor.

maintain continence. There is good pharmacological evidence supporting the view that noradrenaline-mediated contraction of the urethral smooth muscle has an important role (Andersson and Wein, 2004).

Among impairments in urine storage, stress urinary incontinence (SUI) is recognized as one of the most frequently occurring conditions. Deficient urethral or bladder neck closure may result in this condition. However, drug treatments for SUI are scarce, at present (Andersson and Wein, 2004). The density of noradrenergic nerves increases markedly towards the bladder neck and urethra (Gosling *et al.*, 1999). α_1-Adrenoceptors play important roles in both urethral and bladder neck contraction, and may be related to SUI (Andersson and Wein, 2004).

During the voiding phase, prostatic smooth muscle tone contributes to outlet resistance regulation. Benign prostatic hyperplasia (BPH) is a common enlargement of the prostate gland that may lead to bladder outlet obstruction, and lower urinary tract symptoms. BPH is currently recognized as a target for pharmacotherapy utilizing α_1-adrenoceptor antagonists. In BPH patients, enlargement of the prostate increases bladder outlet resistance and thereby impedes physiological voiding. α_1-Adrenoceptor antagonists are believed to inhibit contraction of the prostate and urethra, and as a result, α_1-adrenoceptor antagonists such as tamsulosin, alfuzosin and silodosin effectively reduce resistance to urinary flow and are now clinically used in BPH patients (Lefevre-Borg *et al.*, 1993; Cooper *et al.*, 1999; Ruffolo and Hieble, 1999; Takeda *et al.*, 1999; Chapple, 2001; Andersson, 2002; Michel and Vrydag, 2006).

The functional roles of α_1-adrenoceptors have been highlighted through the study of their roles in lower urinary tract physiology. These are supported by numerous studies including quantification of mRNAs, anatomical localization of mRNAs, or of receptors demonstrated by ligand-binding studies (Table 1). On the other hand, functional studies have shown discrepancies between the native α_1-adrenoceptors found in the lower urinary tract (so-called α_{1L}-adrenoceptor) and the classical α_1-adrenoceptors (α_{1A}, α_{1B} and α_{1D}; receptor nomenclature follows Alexander *et al.*, 2011) (Table 2). Subsequent studies revealed the origin of these unique α_1-adrenoceptors in the lower urinary tract (Ford *et al.*, 1997; Gray *et al.*, 2008; Muramatsu *et al.*, 2008; Nishimune *et al.*, 2010b). In this review, we will introduce recent progress in the study of α_1-adrenoceptors in the lower urinary tract as an example of the importance of phenotype pharmacology (Kenakin, 2003; Muramatsu *et al.*, 2005; 2008; Nelson and Challiss, 2007; Su *et al.*, 2008).

α_1-Adrenoceptor mRNAs in the lower urinary tract

Three cDNAs for distinct α_1-adrenoceptor subtypes (α_{1a}, α_{1b} and α_{1d}) have been cloned, and their pharmacological phenotypes are consistent with pharmacological characteristics of α_1-adrenoceptors found in many native tissues (Lomasney *et al.*, 1991; Hieble *et al.*, 1995) (Table 2). In the lower urinary tract, the mRNA and protein for these three subtypes have been demonstrated (Table 1) (Michelotti *et al.*, 2000; Michel and Vrydag, 2006; Nishimune *et al.*, 2010a). Among them, the α_{1a}-adrenoceptor mRNA is the most form in the lower urinary tract of many species (Table 1). Data from human studies indicate that the relative abundance ratio of α_{1a}- : α_{1b}- : α_{1d}-adrenoceptor mRNA is approximately 70:0:30 in the prostate and 90~100:0:0~10 in the urethra (Price *et al.*, 1993; Faure *et al.*, 1994; Nasu *et al.*, 1996; 1998; Michel and Vrydag,

Table 1

α_1-Adrenoceptor subtypes in lower urinary tract

Tissue	Species	mRNA	Binding	Function
Prostate	Rat	α_{1a}[1,2] α_{1a}, α_{1b}, α_{1d}[3-5]	α_{1A}[12] α_{1A}, α_{1B}[13]	α_{1A}[12,18,19] α_{1L}[13]
	Rabbit	α_{1a}[6]	α_{1A}, α_{1L}[14]	α_{1A}[20–22] α_{1L}[14,23–25]
	Human	α_{1a}[7] $\alpha_{1a} > \alpha_{1d}$[8–11]	α_{1A}[15] α_{1A}, α_{1B}[16] α_{1A}, α_{1L}[17]	α_{1A}[15,18,19,26–29] α_{1L}[15,17,30]
Urethra	Rat	$\alpha_{1a} > \alpha_{1b} > \alpha_{1d}$[31]		α_{1L}[33]
	Rabbit		α_{1A}[15]	α_{1A},[15,34] α_{1L}[25,35]
	Human	$\alpha_{1a} > \alpha_{1d}$[32]		α_{1L}[36]
Bladder neck	Rat	$\alpha_{1a} > \alpha_{1d} > \alpha_{1b}$[1,37,38]	α_{1A}[18,38]	α_{1A}[33,41]
	Rabbit			α_{1A}[18,34] α_{1L}[25,35,42,43]
	Human	$\alpha_{1d} > \alpha_{1a}$[37] α_{1a}, α_{1b}, α_{1d}[39]	$\alpha_{1D} > \alpha_{1A}$[35] α_{1A}, α_{1B}, α_{1D}[40]	α_{1A}[18] α_{1L}[36]

mRNA: subtypes identified from RT-PCR studies or RNase protection assays.
Binding: subtypes detected by radioligand binding assay with tissue homogenates or membrane preparations, but in References 14 and 17, tissue segments were also used in binding assay.
Function: data obtained from bioassay where the contractile responses to noradrenaline or other α_1-agonists were examined.
According to the standard nomenclature (Alexander *et al.*, 2011), the term α_{1A} is used to refer to receptor subtype. In this review, we will use the term only for receptor phenotype in order to focus on phenotypic variation (α_{1A} or α_{1L}) arising from the same gene (*Adra1A*). To refer to receptor polypeptide, we instead use the term α_{1a}.
The superscripted numbers refer to the relevant reference and are shown here in short form; the full form is provided in the usual list of references below.
1. Scofield *et al.* (1995); 2. Walden *et al.* (1997); 3. Rokosh *et al.* (1994); 4. Homma *et al.* (2000); 5. Foster *et al.* (2004); 6. Piao *et al.* (2000); 7. Hirasawa *et al.* (1993); 8. Price *et al.* (1993); 9. Faure *et al.* (1994); 10. Tseng-Crank *et al.* (1995); 11. Nasu *et al.* (1996); 12. Yazawa and Honda (1993); 13. Hiraoka *et al.* (1999); 14. Su *et al.* (2008); 15. Testa *et al.* (1993); 16. Michel *et al.* (1996); 17. Morishima *et al.* (2007); 18. Chang *et al.* (2000); 19. Lagu *et al.* (2000); 20. Honda *et al.* (1985); 21. Delaflotte *et al.* (1996); 22. Yamagishi *et al.* (1996); 23. Hiraoka *et al.* (1995); 24. Leonardi *et al.* (1997); 25. Van der Graaf *et al.* (1997); 26. Forray *et al.* (1994); 27. Marshall *et al.* (1995); 28. Chess-Williams *et al.* (1996); 29. Eltze *et al.* (2001); 30. Ford *et al.* (1996); 31. Yono *et al.* (2004); 32. Nasu *et al.* (1998); 33. Muramatsu *et al.* (unpublished observation); 34. Honda and Nakagawa (1986); 35. Deplanne and Galzin (1996); 36. Taki *et al.* (1999); 37. Malloy *et al.* (1998); 38. Hampel *et al.* (2002); 39. Nomiya and Yamaguchi (2003); 40. Levin *et al.* (1988); 41. Lluel *et al.* (2003); 42. Kava *et al.* (1998); 43. Williams *et al.* (1999).

Table 2

α_1-Adrenoceptor subtypes and their pharmacological characterization

Gene	Receptor polypeptide	Phenotype	Affinity (mean pK_B) Prazosin	Silodosin	Tamsulosin	RS-17053
ADRA1A	α_{1a}	α_{1A}	9.6	9.8	10.0	8.7
		α_{1L}	8.0	9.8	10.0	6.3
ADRA1B	α_{1b}	α_{1B}	10.2	8.0	9.3	7.8
ADRA1D	α_{1d}	α_{1D}	10.0	8.4	9.9	7.8

Affinity values are from Morishima *et al.* (2007).

2006). Interestingly, however, the α_{1a}-adrenoceptor mRNA detected at the tissue level disappears in cultures of human prostatic smooth muscle cells, in contrast to the consistent expression of α_{1b}- and α_{1d}-adrenoceptor mRNAs (Boesch *et al.*, 1999). The relative expression of α_{1a}-adrenoceptor mRNA in the lower urinary tract of humans has also been examined by *in situ* hybridization studies. In the human prostate, α_{1a}-adrenoceptor mRNA was mainly detected in the stroma,

including smooth muscle cells, but not in the glandular epithelium (Walden *et al.*, 1999).

Data regarding the expression of α_1-adrenoceptor mRNA in the human bladder are inconsistent, but it appears that the expression of α_1-adrenoceptor mRNA is extremely low in the bladder (Malloy *et al.*, 1998; Nomiya and Yamaguchi, 2003). In the human detrusor, β (mainly β_3)-adrenoceptors are dominant over α-adrenoceptors, based on the fact that the normal response to noradrenaline is relaxation rather than contraction (Andersson, 1993). Therefore, we can conclude that the functional significance of α-adrenoceptors in the detrusor contraction may be marginal or non-existent (Fig. 1). On the other hand, the density of noradrenergic nerves increases markedly towards the bladder neck, where the smooth muscle receives a dense noradrenergic nerve supply (Gosling *et al.*, 1999). In the human bladder, the predominant expression of α_{1a}-adrenoceptor mRNA in the dome, trigone and base has also been reported (Walden *et al.*, 1997).

Thus, the mRNA data in human urinary tract indicate that the α_{1a}-adrenoceptor polypeptide is a potential target for pharmacotherapy in patients with BPH and SUI.

α_1-Adrenoceptor pharmacological anomalies in the lower urinary tract

The classical α_1-adrenoceptors (α_{1A}, α_{1B} and α_{1D}) show high affinity for prazosin (pKi or pK_B > 9) in most binding and functional studies (Lomasney *et al.*, 1991; Hieble *et al.*, 1995; Ford *et al.*, 1996; Theroux *et al.*, 1996; Taniguchi *et al.*, 1999; Suzuki *et al.*, 2000; Israilova *et al.*, 2004) (Table 2). However, the functional α_1-adrenoceptors identified in the lower urinary tract are known to be relatively resistant to prazosin (Muramatsu *et al.*, 2009) (pK_B = ~8) (Table 1). Thus, this pharmacological anomaly, despite the predominance of α_{1a}-adrenoceptor mRNA, caused confusion about the α_1-adrenoceptor subtypes in the lower urinary tract. Because different affinities for antagonists have traditionally been regarded as characteristic pharmacological criteria for defining novel receptors (Kenakin, 1995; Rang, 2006), the anomalous α_1-adrenoceptor showing low affinity for prazosin suggests the existence of a distinctive subtype (or phenotype).

Pharmacological heterogeneity of α_1-adrenoceptors was originally reported by Drew (1985), who first noted a wide variation in the functional affinities for yohimbine and prazosin. This evidence was confirmed in experiments with isolated blood vessels by Flavahan and Vanhoutte (1986), and they proposed two distinct α_1-adrenoceptor subtypes that could be distinguished by their different affinities for both prazosin and yohimbine (α_{1H} and α_{1L} according to their *H*igh or *L*ow affinity for prazosin). This subclassification was subsequently extended by many pharmacologists and in many tissues including the lower urinary tract (Muramatsu *et al.*, 1990; 1991; 1995; Ford *et al.*, 1996; Testa *et al.*, 1997; Stam *et al.*, 1999; Argyle and McGrath, 2000). In this subclassification, the classical α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors are included in the α_{1H} group, and the anomalous α_1-adrenoceptor showing low affinity for prazosin in the lower urinary tract has been reported as the α_{1L}-adrenoceptor (Muramatsu *et al.*, 1991; 1995; Ford *et al.*, 1996; Langer, 1999; Nishimune *et al.*, 2010a). Antagonistic potencies against noradrenaline-induced contractions in rat, rabbit and human prostate are low for prazosin (pK_B = ~8) and α_{1A}-antagonists (5-methylurapidil, WB4101, RS-17053), but are high for silodosin and tamsulosin (Hiraoka *et al.*, 1995; 1999; Ford *et al.*, 1996; Leonardi *et al.*, 1997; Testa *et al.*, 1997; Van der Graaf *et al.*, 1997; Morishima *et al.*, 2007; Su *et al.*, 2008). In contrast to this nomenclature, α_1-adrenoceptors in the lower urinary tract have been also named as α_{1A}-adrenoceptors in many reports (Table 1). Therefore, there has been confusion in defining the functional α_1-adrenoceptor in the lower urinary tract (Table 1), which may in part depend on distinct subclassifications (α_{1H}, α_{1L} vs. α_{1A}, α_{1B}, α_{1D}).

α_2-Adrenoceptor can also be detected at the mRNA and protein levels in urogenital tissues (Yablonsky *et al.*, 1986; Michel and Vrydag, 2006). Therefore, the anomalous characteristics of adrenergic contractions in the lower urinary tract may be associated with the α_2-adrenoceptor. In fact, clonidine (α_2-agonist) can produce a significant contraction in the female rabbit urethra (Larsson *et al.*, 1986). However, clonidine is inactive in the isolated female human urethra, while noradrenaline produces contractions through sites with low affinity for prazosin (Taki *et al.*, 1999). These results show that the α_2-adrenoceptor is not significantly involved in the anomalous contraction in the human urethra (Ruffolo and Hieble, 1999), although there may be relevant species differences in this adrenoceptor.

Identification of α_1-adrenoceptors by radioligand binding assay

Because reliable subtype-specific α_{1a}-adrenoceptor antibodies were recently reported to be unavailable (Jensen *et al.*, 2009), investigation of α_1-adrenoceptors at the protein level are herein summarized from radioligand-binding experiments.

Binding assay with membrane homogenates

Specific binding of [^{3}H]-prazosin, [^{125}I]-HEAT, [^{3}H]-tamsulosin and [^{3}H]-silodosin to α_1-adrenoceptors in membrane preparations of the lower urinary tract has been detected, with the density of binding in the order, prostate > urethra > bladder. In the rat, rabbit and human prostate, the most abundant α_1-adrenoceptor is the α_{1A}-subtype, which has been identified as having high affinity for prazosin, tamsulosin, silodosin and other α_{1A}-selective antagonists (5-methylurapidil and RS-17053) (Tables 1 and 2). Thus, low-affinity sites for prazosin (α_{1L}-adrenoceptors) are not detected in the membrane preparations of lower urinary tract tissues and at subnanomolar concentrations of [^{3}H]-prazosin. The high abundance of the binding sites showing α_{1A}-adrenoceptor profile in the lower urinary tract is in agreement with the mRNA expression data mentioned above.

Three α_1-adrenoceptor cDNAs (including splice variants of α_{1A}-adrenoceptors) were transfected in several cell lines (CHO cells, COS-7 cells, HEK293 cells, HeLa cells) and the pharmacological binding characteristics in the membrane preparations were compared with those in native tissues. The three recombinant α_1-adrenoceptors showed high (subnanomolar) affinity for prazosin (Table 2). The cloned α_{1a}-

adrenoceptor and its splice variants showed the same pharmacological profile as that recognized in the membrane preparations of lower urinary tract (Lomasney *et al.*, 1991; Theroux *et al.*, 1996; Piao *et al.*, 2000; Suzuki *et al.*, 2000; Ramsay *et al.*, 2004).

Binding assay with whole cells and intact tissues

Most radioligand-binding studies conducted to date have involved homogenates or membrane preparations of tissues or cells as a source of receptors. Because tissue/cell homogenization physically disturbs the receptor microenvironment, it may cause changes in some of the pharmacological characteristics of the receptor. Therefore, in order to retain the natural/native receptor conformation by minimizing physical agitation, whole cells or intact tissue segments must be used in binding assays.

Ford and co-workers reported that the pharmacological characteristics of the recombinant α_{1a}-adrenoceptor can vary substantially depending upon assay conditions (Ford *et al.*, 1997; Daniels *et al.*, 1999). In CHO cells expressing the recombinant α_{1a}-adrenoceptor, prazosin and RS-17053 (α_{1A}-selective antagonist) show substantially lower potency against functional responses (phosphatidyl inositol turnover and calcium influx) in intact cells than inhibition of radioligand binding to α_{1a}-adrenoceptors in membrane homogenates. Furthermore, the K_i values for prazosin and RS-17053 as competitive inhibitors of [^{3}H]-prazosin binding to α_{1a}-adrenoceptors in these CHO cells are dependent on assay conditions, with lower affinity observed when binding is conducted under more physiological conditions (culture medium, intact cells, 37°C) than under conditions commonly employed for radioligand binding assays (artificial buffer, membrane homogenates, 20°C). These observations have led to the proposal that the α_{1L}-adrenoceptor may not represent an independent molecular entity, but rather may be an 'affinity state' of the α_{1A}-adrenoceptor that is predominant in lower urinary tract.

Recently, a tissue segment binding method was developed and applied to numerous tissues (Tanaka *et al.*, 2004; Muramatsu *et al.*, 2005). In this method, tissue segments are incubated (without homogenization) in a nutrient medium such as Krebs solution during the course of ligand binding. To identify the α_1-adrenoceptors having low affinity for prazosin, [^{3}H]-silodosin was used instead of [^{3}H]-prazosin, because [^{3}H]-prazosin at subnanomolar concentrations cannot bind sufficiently to α_{1L}-adrenoceptors (Su *et al.*, 2008; Muramatsu *et al.*, 2009). Silodosin and its tritiated radioligand are known to be of equally high affinity for both the α_{1A}- and α_{1L}-adrenoceptors (Murata *et al.*, 1999; Su *et al.*, 2008) (Table 2).

In segments of human, mouse and rabbit prostate, the binding of [^{3}H]-silodosin was biphasically displaced by prazosin, indicating the coexistence of high and low-affinity sites for prazosin, which correspond to α_{1A}- and α_{1L}-adrenoceptors (Morishima *et al.*, 2007; Muramatsu *et al.*, 2008; Su *et al.*, 2008). This result is very different from the results obtained in homogenates, in which a single high-affinity site for prazosin (α_{1A}-adrenoceptor) was detected. The low-affinity site for prazosin in the prostate segments also showed low affinity for some α_{1A}-selective antagonists (RS-17053, 5-methylurapidil), but tamsulosin (which has high affinity for α_{1A}-, α_{1L}- and α_{1D}-subtypes) did not discriminate either the high- or low-affinity sites for prazosin. Similar results were obtained in studies of other tissues in which the α_{1L}-adrenoceptor was identified as a functional receptor (Hiraizumi-Hiraoka *et al.*, 2004; Morishima *et al.*, 2008; Muramatsu *et al.*, 2008; 2009). From these observations, it was suggested that α_{1L}-adrenoceptors (or α_{1L}-phenotype) coexist with α_{1A}-adrenoceptors (or α_{1A}-phenotype) as pharmacologically distinct entities under intact segment conditions, whereas the pharmacological profile of α_{1L}-adrenoceptors converts to the α_{1A}-phenotype upon homogenization.

More recently, this conclusion was again confirmed in a recombinant system (Nishimune *et al.*, 2010b). In the CHO cell line, transfection of α_{1a}-adrenoceptor cDNA predominantly expresses α_{1A}-adrenoceptor phenotype, with an extremely minor proportion of α_{1L}-adrenoceptor (less than 10% of total α_1-adrenoceptor). However, persistent overexpression of the protein, cysteine-rich epidermal growth factor-like domain 1α (CRELD1α), which was found as a potential α_{1a}-adrenoceptor-interacting protein candidate) strongly reduced the population of α_{1A}-adrenoceptor phenotype in CHO cells (Nishimune *et al.*, 2010b). Although mechanisms underlying the interactions between generation of CRELD1α and α_{1A}-adrenoceptors and α_{1L}-adrenoceptors remain unclear, two distinct (α_{1L}-adrenoceptor-dominant and α_{1A}-adrenoceptor-dominant) CHO cell lines were eventually established. Under whole-cell conditions, in contrast to the α_{1A}-adrenoceptor, pharmacological and functional properties of the established α_{1L}-adrenoceptor show low affinity for prazosin and other α_{1A}-adrenoceptor antagonists (5-methylurapidil, RS-17053), and the agonist and antagonist pharmacology is consistent with the profile of the α_{1L}-adrenoceptor identified in the lower urinary tract. Therefore, from these lines of evidence, it may be now concluded that the α_{1L}-adrenoceptor is one of the α_{1a}-adrenoceptor gene products and occurs as an entity distinct from the α_{1A}-adrenoceptor phenotype under conditions when the tissue/cell is kept intact. This conclusion would also explain why α_{1L}-adrenoceptors could not be detected after homogenization but are easily recognized in functional bioassay studies with intact tissue strips (Muramatsu *et al.*, 2009).

Identification of the gene encoding α_{1L}-adrenoceptor

Despite extensive searches at early stages after the proposal of the α_{1L}-adrenoceptor and the subsequent completion of the human genome sequencing project, a distinct gene for the proposed α_{1L}-adrenoceptor has not been identified. Rather, as described above, a close relationship between α_{1L}-adrenoceptor and α_{1A}-adrenoceptor has been considered (Ford *et al.*, 1997; Hiraizumi-Hiraoka *et al.*, 2004; Morishima *et al.*, 2008).

In order to explore this possible link between α_{1A}- and α_{1L}-adrenoceptors, we analysed *in vivo* phenotypes of mice having disrupted alleles of the classical α_1-adrenoceptors (α_{1A}, α_{1B} and α_{1D}) (Muramatsu *et al.*, 2008). The integrity of the α_{1L}-adrenoceptors were confirmed both in terms of ligand

binding properties and contractile function in the *Adra1B*$^{-/-}$ ($\alpha_{1b}^{-/-}$) and *Adra1D*$^{-/-}$ ($\alpha_{1d}^{-/-}$) mice as well as in the wild-type mice. In contrast, both α_{1L}- and α_{1A}-adrenoceptors were completely absent from the *Adra1A*$^{-/-}$ ($\alpha_{1a}^{-/-}$) mice. These results unequivocally demonstrate that both α_{1A}-adrenoceptors and α_{1L}-adrenoceptors are derived from the same *Adra1A* (α_{1a}) gene (Gray *et al.*, 2008; Muramatsu *et al.*, 2008) (Table 2).

Observation of α_{1L}-adrenoceptor distribution by fluorescent probe

Alexa Fluor 488 dye conjugated with silodosin (Alexa-488-silodosin) was recently introduced as a fluorescent probe (Morishima *et al.*, 2010). Alexa-488-silodosin retains the high affinity and selectivity for α_{1A}- and α_{1L}-adrenoceptors as shown by unlabelled silodosin. Histochemical experiments with this fluorescent probe clearly showed that Alexa-488-silodosin binds to the smooth muscle but not the glandular tissue in the human prostate, and that the binding is resistant to low concentrations of prazosin. These results are in good agreement with *in situ* hybridization data showing selective expression of α_{1A}-adrenoceptor mRNA in the stroma of the human prostate (Walden *et al.*, 1999). As the Alexa-488-silodosin can specifically label α_{1L}-adrenoceptors, particularly by co-incubation with low concentrations of prazosin (to mask the α_{1A}-adrenoceptor), this novel molecular probe provides a versatile tool to study α_{1L}-adrenoceptor histochemically (Morishima *et al.*, 2010).

Perspective

As mentioned above, recent progress in this field clearly demonstrates that two distinct α_1-adrenoceptor phenotypes (α_{1A} and α_{1L}) originate from a single α_{1a}-adrenoceptor gene and coexist in some tissues. However, adrenergic contraction in the lower urinary tract is predominantly mediated through α_{1L}-, but not α_{1A}-adrenoceptors (Fig. 1). In general, the affinity values (pKi or pK_B) for prazosin for α_{1L}-adrenoceptors are around 8, but recent studies reveal a further variation in prazosin affinity (pKi or pK_B = 6.3–8.5) at various α_{1L}-adrenoceptors in many tissues and species (Muramatsu *et al.*, 2009). This is reminiscent of the original question on α_1-adrenoceptors (Drew, 1985), suggesting further heterogeneity in α_{1L}-adrenoceptor pharmacology. At present, the mechanisms underlying the expression of α_{1L}-adrenoceptor phenotype and its functional predominance in several tissues remain unknown. However, it is likely that the expression of divergent α_{1L}-adrenoceptor phenotypes is strongly dependent on any modification of the tissues of various species, rather than a simple variation of the α_{1a}-adrenoceptor protein or additional subtypes (Muramatsu *et al.*, 2009; Nishimune *et al.*, 2010a).

Recently, ample evidence has been accumulating suggesting that antagonist affinity is not necessarily constant at a given receptor expressed in different tissues/cells and examined under different assay conditions (Kenakin, 2003; Baker and Hill, 2007; Nelson and Challiss, 2007; Muramatsu *et al.*, 2008). The α_{1L}-adrenoceptor exemplifies this type of variable affinity, as after homogenization, the phenotype changed from α_{1L} into α_{1A}. It is likely that tissue integrity is an important factor to determine receptor properties (Su *et al.*, 2008; Muramatsu *et al.*, 2009). Therefore, we may have to re-evaluate pharmacodynamic and pharmacokinetic effects of currently used drugs, and to reconstruct drug development strategies. Now, α_1-antagonists, such as tamsulosin, silodosin, alfuzosin, are clinically used in BPH patients. According to the evidence mentioned above, these antagonists appear to act mainly on functional α_{1L}-adrenoceptors in the lower urinary tract, but are not specific for the α_{1L}-adrenoceptor. The α_{1L}-adrenoceptors in the female urethra may be a new target in therapy for SUI. For this purpose, two α_1-adrenoceptor agonists (NS-49 and Ro 115-1240) have been developed. However, these compounds are full agonists of the α_{1A}-adrenoceptor or partial agonists of the $\alpha_{1A/L}$-adrenoceptor (Obika *et al.*, 1995; Blue *et al.*, 2004; Musselman *et al.*, 2004). Thus, more selective or specific drugs against α_{1L}-adrenoceptors may lead to improved uroselectivity.

Conclusion

Since the successful cloning of most receptors, it has become possible to elucidate numerous physiological responses by genome-based subtype (genotypes). However, there are still some unique phenotypes showing distinct pharmacology in native tissues. The α_{1L}-adrenoceptor is representative of this group, and originates from the α_{1a}-adrenoceptor gene together with the α_{1A}-adrenoceptor phenotype. In this review, we propose that different phenotypes are expressed from a single gene in native tissues ('one gene-multiple phenotypes theory'), which may explain the long controversy regarding some putative receptors, such as α_{1L}-adrenoceptor in the lower urinary tract, and further highlights phenotype-dependent pharmacology ('phenotype pharmacology').

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Conflict of interest

The authors declare no competing financial interests.

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