α_1 -Adrenoceptors and muscarinic receptors in voiding function – binding characteristics of therapeutic agents in relation to the pharmacokinetics

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In vivo and *ex vivo* binding of α_1 -adrenoceptor and muscarinic receptors involved in voiding function is reviewed with therapeutic agents (α_1 -adrenoceptor antagonists: prazosin, tamsulosin and silodosin; and muscarinic receptor antagonists: oxybutynin, tolterodine, solifenacin, propiverine, imiafenacin and darifenacin) in lower urinary tract symptoms. This approach allows estimation of the inhibition of a well-characterized selective (standard) radioligand by unlabelled potential drugs or direct measurement of the distribution and receptor binding of a standard radioligand or radiolabelled form of a novel drug. In fact, these studies could be conducted in various tissues from animals pretreated with radioligands and/or unlabelled novel drugs, by conventional radioligand binding assay, radioactivity measurement, autoradiography and positron emission tomography. *In vivo* and *ex vivo* receptor binding with α_1 -adrenoceptor antagonists and muscarinic receptor antagonists have been proved to be useful in predicting the potency, organ selectivity and duration of action of drugs in relation to their pharmacokinetics. Such evaluations of drug–receptor binding reveal that adverse effects could be avoided by the use of new α_1 -adrenoceptor antagonists and muscarinic receptor antagonists for the treatment of lower urinary tract symptoms. Thus, the comparative analysis of α_1 -adrenoceptor and muscarinic receptor binding characteristics in the lower urinary tract and other tissues after systemic administration of therapeutic agents allows the rationale for their pharmacological characteristics from the integrated viewpoint of pharmacokinetics and pharmacodynamics. The current review emphasizes the usefulness of *in vivo* and *ex vivo* receptor binding in the discovery and development of novel drugs for the treatment of not only urinary dysfunction but also other disorders.

Introduction

Lower urinary tract symptoms, characterized by an increased frequency of micturition, urgency, urge incontinence and urinary obstruction, are very common in the geriatric population, a group that is rapidly increasing in number [1–4]. α_1 -Adrenoceptor antagonists and muscarinic receptor antagonists are widely used as first-line therapy for lower urinary tract symptoms because of benign prostatic hyperplasia (BPH) and overactive bladder (OAB) [5–8]. Prazosin produces some side effects, such as orthostatic hypotension and dizziness, which are ascribed mainly to a reduction in peripheral resistance mediated

through a blockade of vascular α_1 -adrenoceptors. Tamsulosin and silodosin antagonize α_1 -adrenoceptor-mediated contractile responses to a greater extent in prostate than in vascular tissues [9–12]. Such selectivity of α_1 -adrenoceptor-mediated responses in the lower urinary tract was not seen with prazosin. α_1 -Adrenoceptors are classified into several subtypes [13–15]. The prostate contains predominantly α_{1A} - and α_{1L} -adrenoceptors, and the α_{1L} -adrenoceptor seems to be a variant of the α_{1A} -adrenoceptor subtype [15]. *In vitro* radioreceptor binding and pharmacological studies demonstrate that prazosin and terazosin are nonselective antagonists of α_1 -adrenoceptor subtypes, while tamsulosin and silodosin

are relatively selective antagonists of the α_{1A} -adrenoceptor subtype.

While muscarinic receptor antagonists have proved effective in patients with OAB, they are also associated with anticholinergic side effects, including dry mouth, constipation, somnolence and blurred vision, because the muscarinic receptor mediates the excitatory and inhibitory actions of acetylcholine in the central and peripheral nervous systems [7]. Dry mouth is the most common of these complaints and decreases quality of life. Therefore, numerous studies involving muscarinic receptor antagonists to treat OAB have focused on targeting the urinary bladder rather than the salivary gland. The incidence of side effects of muscarinic receptor antagonists on the central nervous system (CNS) is generally lower than that of dry mouth, but such effects may be of great concern in elderly patients because of an increase in blood–brain barrier (BBB) permeability with ageing [8, 16]. In this connection, clinical studies demonstrate increased cognitive sensitivity to scopolamine [17, 18] and a reduced density of brain muscarinic receptors in the elderly [19]. Therefore, it is important to evaluate the bladder selectivity of muscarinic receptor antagonists used to treat OAB for optimal medication.

This review article focuses on *ex vivo* and *in vivo* receptor binding of α_1 -adrenoceptor antagonists and muscarinic receptor antagonists in the lower urinary tract and CNS, and discusses the rationale of its usefulness for the pharmacological characterization of therapeutic agents used for lower urinary tract symptoms.

Characterization of *in vivo* drug–receptor binding

Currently, a number of novel drugs exhibiting target organ specificity, receptor subtype selectivity and long duration of action have been developed to reduce side effects and improve patient compliance. α_1 -Adrenoceptors and muscarinic receptors in the lower urinary tract and the receptor binding of therapeutically used agents have been extensively characterized by *in vitro* assay techniques, such as radioreceptor binding assays and the measurement of mRNA and protein expression, in tissue membrane preparations and in recombinant cells expressing certain receptor subtypes [20–22]. However, even if these agents display high affinity for a certain receptor subtype in the target organ *in vitro*, such receptor binding characteristics may not necessarily assure pharmacological specificity *in vivo* because various pharmacokinetic and pharmacodynamic factors are not taken into account [23] (Figure 1).

Pharmacokinetics includes the absorption, distribution, excretion and metabolism of drugs, while pharmacodynamics includes the affinity of drugs for receptors, signal transduction and homeostatic mechanisms [23]. The drug–receptor interaction results in a measured effect, and the magnitude of the interaction depends on the concentration of the drug in the biophase and further on its affinity for the receptors. The biophase concentration depends not only on the amount of drugs given, but also on pharmacokinetic factors. Inasmuch as *in vivo*

Figure 1

Schematic representation of *in vivo* drug–receptor binding in relation to pharmacokinetics and pharmacodynamics. This modified figure is reproduced from reference [23] with permission

Figure 2

Schematic representation of *in vivo* drug-receptor binding characteristics in target and nontarget tissues, and physiological, pharmacokinetic and pharmacodynamic factors affecting *in vivo* drug-receptor binding. BBB, blood-brain barrier

pharmacological effects may be complicated by various physiological, pharmacokinetic and pharmacodynamic factors (Figure 2), the *in vivo* analysis of drug-receptor binding allows more practical information for drug therapy to assess comparatively the extent and time course of drug-receptor binding in target tissues and nontarget tissues in physiological conditions. The most frequent approach is to study the extent to which an unlabelled drug inhibits specific binding of a well-characterized selective radioligand. The alternative direct approach is to radiolabel a new potential drug and to trace its uptake, distribution and binding in tissues. In these approaches, the binding characteristics (degree and time course) of the novel drug on the receptors in the target and nontargeted tissues can be assessed simultaneously. The demonstration of a quantitative relationship between drug binding *in vivo* and drug effects in patients is used to validate targets for drug action, to correlate pharmacological and physiological effects and to optimize clinical treatment.

α_1 -Adrenoceptor binding in the prostate

The α_1 -adrenoceptor binding of [3 H]prazosin, [3 H]tamsulosin and [3 H]silodosin after an intravenous injection is widely distributed in various tissues, including the pros-

tate, of rats [24–28]. There was a close correlation in intravenous dose ranges between the *in vivo* α_1 -adrenoceptor binding activities of these agents in the rat prostate and their functional activities in the lower urinary tract [12, 27–29], indicating that [3 H]prazosin, [3 H]tamsulosin and [3 H]silodosin label pharmacologically relevant α_1 -adrenoceptors in the prostate in physiological conditions. In fact, [3 H]tamsulosin may be a suitable radioligand for the *in vivo* labelling of α_1 -adrenoceptors in various tissues, because of its extremely low level of nonspecific binding. The parameters for *in vivo* [3 H]tamsulosin binding could be estimated easily in rat tissues, and there is a similarity between *in vitro* and *in vivo* binding parameters for this radioligand in rat tissues [27].

In rats, the α_{1A} -adrenoceptor subtype exists predominantly in the prostate and submaxillary gland, while the α_{1B} -subtype is predominant in the spleen and liver [30–33]. There was a significant difference between [3 H]tamsulosin or [3 H]silodosin and [3 H]prazosin in the degree and time course of *in vivo* α_1 -adrenoceptor binding in rat tissues [25–28]. The α_1 -adrenoceptor binding of [3 H]tamsulosin and [3 H]silodosin was significantly longer-lasting in the prostate and submaxillary gland than in the spleen, lung and kidney. These results are consistent with the *ex vivo* observation that the oral administration of tamsulosin and silodosin, despite a rapid decline in plasma concentration, causes sustained occupancy of α_1 -adrenoceptors in the rat prostate and submaxillary gland, but not in the spleen

Figure 3

In vivo specific binding of [³H]prazosin, [³H]tamsulosin and [³H]silodosin in the prostate, submaxillary gland, aorta and spleen of rats after the intravenous injection. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 significantly different from the value of [³H]prazosin

[34, 35]. Sustained blockade by silodosin of prostatic α_1 -adrenoceptors is consistent with a functional study showing a prolonged inhibition of phenylephrine-induced intraurethral pressure in rats [12], demonstrating high affinity for the α_1 -adrenoceptor subtype in the prostate *in vivo*.

The *in vivo* α_1 -adrenoceptor binding of [³H]tamsulosin and/or [³H]silodosin in the prostate and submaxillary gland was higher than that for [³H]prazosin (Figure 3) [25–28]. In contrast, the α_1 -adrenoceptor binding of [³H]tamsulosin and [³H]silodosin compared with [³H]prazosin was significantly lower in the spleen, heart and liver of rats. Thus, it is likely that tamsulosin and silodosin bind preferentially to α_{1A} -adrenoceptors compared with α_{1B} -adrenoceptors *in vivo*. Michel and Goepel [36] found that among human α_1 -adrenoceptor subtypes, [³H]tamsulosin labelled fewer sites than [³H]prazosin for α_{1B} - but more sites for α_{1A} - and α_{1D} -adrenoceptors *in vitro*. Also, the low concentration of [³H]prazosin may not capture the α_{1L} -site. Thus, these issues should be taken into account in the interpretation of data obtained with three different radioligands.

[³H]Tamsulosin and [³H]silodosin exhibit essentially similar characteristics of *in vivo* α_1 -adrenoceptor binding in rat tissues, the only difference being less binding capacity for [³H]silodosin than [³H]tamsulosin in the aorta and spleen (Figure 3) [25–28]. This indicates that [³H]silodosin compared with [³H]tamsulosin may exhibit considerably lower affinity for the α_1 -adrenoceptor subtype in the aorta and spleen. α_1 -Adrenoceptor-agonist-induced contraction of the rat aorta is mediated primarily via the α_{1D} -adrenoceptor subtype [37, 38], and tamsulosin, but not silodosin, exhibits high affinity for the α_{1D} -subtype [39–41]. In addition, the α_{1B} -adrenoceptor binding affinity of silodosin was shown to be much lower than that of tamsulosin

in vitro [32, 41]. These observations are associated with the difference between these radioligands in the *in vivo* α_1 -adrenoceptor binding. Silodosin seems to be a highly selective antagonist for the α_{1A} -adrenoceptor subtype *in vivo*. Notably, the absence of *in vivo* α_1 -adrenoceptor binding of [³H]prazosin, [³H]tamsulosin and [³H]silodosin in the cerebral cortex may be as a result of the limited brain distribution because of poor permeability through the BBB [27, 28]. This idea is supported by the *ex vivo* observation that there is little alteration in α_1 -adrenoceptor binding parameters in brain tissues of rats after the oral administration of prazosin and tamsulosin [34, 35]. Thus, it seems unlikely that these agents have any pharmacological effects on the CNS.

Muscarinic receptor binding in the bladder

Oxybutynin was widely used to treat OAB, but its use was often limited by systemic side effects, such as dry mouth, blurred vision, constipation and tachycardia, which appear frequently in patients receiving oral oxybutynin [42]. To overcome these drawbacks, a controlled release dosage form and a transdermal therapeutic system were developed and clinically used, with the advantage of a lower incidence of adverse effects than the immediate release form in patients with OAB [43–45]. These clinical observations coincide with the *ex vivo* data that oral but not transdermal oxybutynin produced a long-lasting blockade of muscarinic receptors in the salivary gland [46]. Furthermore, the antagonism by oral oxybutynin, unlike transdermal oxybutynin, was characterized by a marked suppression of the maximal response of pilocarpine-induced salivation. Taking account of the fact that muscarinic receptor binding affinity of the active metabolite, *N*-desethyl-oxybutynin (DEOB), was greater in the salivary gland than in the bladder [47, 48], the formation and tissue accumulation of a significant amount of DEOB may be responsible for the long-lasting occupation of exocrine receptors after oral oxybutynin treatment. Additionally, it is assumed that pharmacokinetic characteristics of the controlled release form of oxybutynin, such as substantially less fluctuation in the plasma oxybutynin levels, bring about a significant difference in exocrine receptor binding characteristics from those of oral oxybutynin.

Tolterodine, a potent antimuscarinic agent, displays favourable tissue selectivity for the urinary bladder compared with the salivary glands [49–51]. Tolterodine is extensively metabolized in the liver to form an active metabolite, 5-hydroxymethyl metabolite (5-HM) [50, 51]. In *in vitro* experiments, tolterodine, 5-HM and solifenacin displayed concentration-dependent binding of muscarinic receptors in the bladder and salivary gland [52, 53]. Oral administration of tolterodine and solifenacin compared with oxybutynin resulted in relatively slower and longer-

lasting binding in the bladder than in the salivary gland [23, 52–54]. Furthermore, significant receptor binding activity by a lower dose of tolterodine was also observed only in the bladder. Therefore, oral tolterodine binds more selectively to muscarinic receptors in the bladder than in the salivary gland. Tolterodine was significantly weaker than oxybutynin in inhibiting pilocarpine-induced salivation in mice [53]. Considering that most of the administered dose in mice receiving oral [^{14}C]tolterodine was preferentially distributed to organs such as the urinary bladder, liver and kidney [55], the plausible mechanism for the bladder receptor selectivity by oral tolterodine may be closely associated with the preferential distribution of this drug and 5-HM in the tissue.

Propiverine is an effective drug for urinary incontinence because of detrusor overactivity, with a moderate incidence of adverse events [56, 57]. The *in vitro* muscarinic receptor binding activity of propiverine and its N-oxide metabolite [DPr-P-4(N $\rightarrow$ O)] is roughly equipotent. Following oral administration of propiverine, there was relatively selective and longer-lasting binding of muscarinic receptors in the bladder compared with the salivary gland [58–60]. A high concentration of DPr-P-4(N $\rightarrow$ O) was detected in the bladder tissue of rats orally administered propiverine, indicating a specific distribution in the target organ. The intravesical instillation of DPr-P-4(N $\rightarrow$ O) produced significant muscarinic receptor binding in the rat bladder [60]. Thus, DPr-P-4(N $\rightarrow$ O) may contribute greatly to the relatively selective and long-lasting occupation of bladder muscarinic receptors after the oral administration of propiverine.

Imidafenacin, a potent M_1 - and M_3 -subtype-selective antagonist, is now used clinically in Japan for the treatment of OAB [61, 62]. Clinical studies indicated a favourable efficacy-to-side-effect ratio of imidafenacin in patients with OAB [63, 64]. The preferential muscarinic receptor binding in the bladder over the salivary gland, heart, colon, lung and brain of this agent is demonstrated by *ex vivo* receptor binding (Figure 4) [65]. The oral administration of imidafenacin at low doses caused a more selective and longer-lasting binding to muscarinic receptors in the bladder than in other tissues. Pharmacokinetic data show that the orally administered imidafenacin distributed at a higher concentration in the bladder than in the serum or submaxillary gland of rats. Further more, a significant level of imidafenacin was detected in the urine of rats treated orally with this agent. The intravesical instillation of imidafenacin resulted in significant binding of bladder muscarinic receptors. Thus, it is likely that imidafenacin administered orally distributes predominantly to the bladder and binds, in a more selective and longer-lasting manner, to muscarinic receptors in this tissue than in other tissues, such as the submaxillary gland. Imidafenacin excreted in urine is assumed to play an important role in the pharmacokinetic and pharmacological selectivity.

Assessment of brain muscarinic receptor binding by positron emission tomography

Positron emission tomography (PET) is an imaging technique to monitor the delivery of tracers labelled with positron emitters. A variety of probes have been labelled to measure biochemical and physiological parameters in the CNS, such as glucose and oxygen metabolism, protein synthesis, blood flow and neurotransmitter receptors. A higher resolution PET system was applied for the *in vivo* imaging of neuronal activation and plasticity in the rodent brain [66, 67]. PET is a powerful noninvasive technique that can be used to examine the distribution and receptor binding of radioligands or novel drugs in the CNS [68, 69]. When the receptor binding of drugs is assessed by use of PET, several factors, such as the endogenous neurotransmitter and/or the metabolism of radioligands, should be carefully taken into account, otherwise serious mistakes could be arise [68]. Therefore, PET results should be always assessed in the combination with data obtained by other analytical methods, such as *ex vivo* binding assay and pharmacological procedures. Unfortunately, receptor binding measurement by PET in most peripheral tissues, including the lower urinary tract, is not currently successful.

The *in vivo* imaging of brain receptors by autoradiography (ARG) and PET allows the precise localization of muscarinic receptors and the pharmacological characterization of muscarinic receptor antagonists. The testing of muscarinic receptor antagonists for side effects on the CNS is important. Yoshida *et al.* [70] noninvasively characterized muscarinic receptor occupancy in the rat brain after the systemic injection of oxybutynin, darifenacin and imidafenacin using PET, comparing the results with those of *in vivo* ARG and *ex vivo* radioreceptor assays. In the PET study using rats, the intravenous injection of oxybutynin, but not darifenacin or imidafenacin, at pharmacological doses decreased the binding potential of (+)-N-[^{11}C]methyl-3-piperidyl benzilate ([^{11}C](+)-3-MPB), a PET ligand of muscarinic receptors, in the cerebral cortex and corpus striatum in a dose-dependent manner (Figure 5) [70]. Likewise, in the receptor ARG analysis, oxybutynin decreased the binding of [^{11}C](+)-3-MPB in each region of the brain in a dose-dependent manner, whereas darifenacin and imidafenacin had little effect (Figure 6). Also, a PET study in conscious rhesus monkeys showed that the oral administration of oxybutynin at clinically relevant doses resulted in significant (40–60%) occupancy of muscarinic receptors in the brain (SY, YI and HT, unpublished observations). In agreement with the PET results, short-term and chronic administration of oxybutynin in elderly subjects resulted in mild cognitive dysfunction [71, 72]. Consequently, the results obtained by ARG and PET [70, 73] confirm *ex vivo* binding data for oxybutynin, darifenacin and imidafenacin on muscarinic receptors [23, 65, 74] and seem to be

Figure 4

Muscarinic receptor binding (increase in the dissociation constant, K_d , for specific [3 H]NMS binding) in the bladder, submaxillary gland, heart, colon, lung and brain of rats 1–12 h after the oral administration of imidafenacin [0.25 (A), 0.50 (B) or 2.0 mg kg⁻¹ (C)]. Control (□); 1 h (■); 3 h (▨); 6 h (▩); 9 h (▤); 12 h (■)

responsible for clinical observations indicating that darifenacin and imidafenacin might have fewer adverse effects on the CNS than oxybutynin [63, 71, 72, 75].

The intravenous injection of propiverine, tolterodine and solifenacin significantly decreased *in vivo* specific binding of [11 C](+)-3-MPB in each region of the brain in rats in a dose-dependent manner [73]. Their estimated RO_{50} values (doses at which a half-maximal receptor occupancy is obtained) are similar to i.v. doses inhibiting learning/memory behaviour in rats [76]. Thus, these values could be regarded as an index of CNS pharmacological effects fol-

lowing the blockade of brain muscarinic receptors. The dose ratios (RO_{50}/ID_{50}) of muscarinic receptor antagonists for brain receptor occupancy to the inhibitory potency of increases in intravesical pressure, which reflect *in vivo* pharmacological selectivity for the urinary bladder over the brain, are relatively large for solifenacin, tolterodine and propiverine compared with oxybutynin. In fact, the incidence of CNS side effects of tolterodine was lower than that of oxybutynin in patients with OAB [77, 78]. Thus, the selectivity for the urinary bladder over the brain seems to be relatively high for new-generation muscarinic receptor

Figure 5

(A) Typical positron emission tomography images fused with computed tomography images in the brain of rats injected intravenously with [¹¹C](+)-3-MPB. The colour coding represents linear pseudocolouring of phosphor imager signal units (% of maximum). This modified figure was reproduced from reference [70] with permission

antagonists, such as imidafenacin, suggesting a low likelihood of CNS side effects at pharmacological doses to treat OAB.

Muscarinic receptor antagonists must first cross the BBB to distribute into the brain and to occupy the CNS receptors. The observed difference among muscarinic receptor antagonists in the degree of uptake and binding to muscarinic receptors in the brain may depend on their ability to permeate the BBB. The passive penetration of the BBB is dependent principally on physicochemical properties [77], and thus its small size and molecular characteristics (high lipophilicity and neutral polarity) make oxybutynin likely to cross the BBB. In contrast, both darifenacin and imidafenacin have moderate polarity and

low lipophilicity, suggestive of lower permeability. In addition, darifenacin is considered a substrate of P-glycoprotein, an active-transport system that carries this agent back across the BBB [79].

The muscarinic receptor subtype selectivity of muscarinic receptor antagonists may also be involved in the risk of side effects. Although all five muscarinic receptor subtypes are expressed in the CNS [80], the M₁ receptor, in particular, is considered to play a crucial role in modulating cognitive function [81]. Oxybutynin and imidafenacin are selective for both M₁ and M₃ receptors, while darifenacin is selective of M₃ receptors [22, 61, 82]. Thus the side effect of oxybutynin on the CNS during OAB treatment may be attributed to the selectivity for the M₁ receptor in addition

Figure 6

(A) Typical autoradiographic images in the brain of rats injected intravenously with [¹¹C](+)-3-MPB. (B) Effects of different doses of oxybutynin (0.1–1.0 mg kg⁻¹), darifenacin (0.1–1.0 mg kg⁻¹) and imidafenacin (0.01–0.1 mg kg⁻¹) on autoradiographic images of [¹¹C](+)-3-MPB in the rat brain. The colour coding represents linear pseudocolouring of phosphor imager signal units (% of maximum). This modified figure was reproduced from reference [70] with permission

to the ability to permeate the BBB. In contrast, imidafenacin exerts little influence on cognitive function [62] and on brain muscarinic receptor binding [70], possibly because of the insignificant distribution of imidafenacin in the CNS.

Comparison of *in vivo* and *ex vivo* receptor binding assay

For *in vivo* study with radioligands, rats and mice can generally be used, but mice may be more convenient for the *in vivo* measurement of the distribution and receptor binding

in tissues by use of a small amount of the ligand. In contrast, rats may be useful for the *ex vivo* receptor binding assay of novel drugs after systemic administration because of the larger amount of tissues. The tissue distribution and direct receptor binding *in vivo* could be estimated by the determination of radioactivity in various tissues after the i.v. injection of a well-characterized radioligand for a certain receptor (e.g. [³H]NMS for muscarinic receptors) or radiolabelled form of a novel drug. The technique using PET could also monitor the delivery and receptor binding of tracer labelled with positron emitters. Using these methods, we could assess the *in vivo* receptor binding

activity and selectivity of nonlabelled novel drugs pre-treated with high sensitivity by the competition with radioligand. However, in this case, the definition of nonspecific binding and the pharmacokinetics, such as the metabolism and protein binding of the radioligand, should be cautiously considered for the exact estimation of the receptor binding. Furthermore, it should be kept in mind that such *in vivo* methodology represents receptor binding characteristics with only a trace (not a pharmacological) dose of the radioligand.

The *ex vivo* binding assay using a well-characterized (standard) radioligand in tissues removed after the systemic administration of nonlabelled novel drugs allows investigation of the receptor binding characteristics (extent, duration, etc.) at pharmacological doses. In fact, the competitive or noncompetitive mode of receptor binding of novel drugs could be predicted by the changes of binding parameters (dissociation constant or maximal binding sites) of radioligand. However, there is a possibility that *ex vivo* study underestimates the amount of receptor binding of the administered drugs in tissues because of some dissociation of the receptor-bound drugs from the receptor sites during the preparation of tissue homogenates. In the *ex vivo* study compared with *in vivo* study, the dissociation of the receptor-bound drugs should be cautious. Most importantly, the precision of *in vivo* and *ex vivo* receptor binding data could be confirmed by the consistency with functional data on drugs determined by *in vivo* pharmacological methods, particularly in terms of pharmacological specificity, such as effective dosages, time course, duration and tissue selectivity. In fact, essentially similar results are obtained in the *in vivo* and *ex vivo* measurement of receptor binding of both α_1 -adrenoceptor antagonists and muscarinic receptor antagonists used to treat lower urinary tract symptoms, and they generally corroborate pharmacological results [20–23, 25–28, 34, 35, 46, 48, 52–54, 59, 60, 65, 70, 73, 74, 82, 83].

Future directions

The *in vivo* drug–receptor binding may be largely influenced by physiological factors, such as the intrinsic activity of nervous and hormonal systems. It is speculated that pathological conditions such as BPH and OAB affect *in vivo* binding of α_1 -adrenoceptor antagonists and muscarinic receptor antagonists. Significant alterations of pharmacological receptors in the lower urinary tract of experimental animal models with lower urinary tract symptoms were revealed [84–87]. Also, there were significant alterations of central and peripheral muscarinic receptors in tissues of muscarinic receptor knockout mice [80, 88]. Muscarinic receptor subtype knockout mice may be useful to characterize the *in vivo* muscarinic receptor binding of muscarinic receptor antagonists, because muscarinic receptor binding in the bladder of M_2 knockout mice was significantly

greater for oral solifenacin than oral oxybutynin [83]. The extensive analysis of *in vivo* drug–receptor binding characteristics in pathological states such as BPH and OAB may be of great importance for the preclinical evaluation of therapeutic agents in lower urinary tract symptoms.

Conclusions

The *in vivo* and *ex vivo* techniques using ARG and PET to measure drug–receptor binding are useful in predicting the potency, organ selectivity and duration of action of drugs in relation to their pharmacokinetics. Such evaluations of drug–receptor binding reveal that adverse effects could be avoided by the use of relatively new α_1 -adrenoceptor antagonists and muscarinic receptor antagonists for the treatment of lower urinary tract symptoms. The comparative analysis of α_1 -adrenoceptor and muscarinic receptor binding characteristics in the lower urinary tract and other tissues after the systemic administration of therapeutic agents allows the rationale for their pharmacological characteristics from the integrated viewpoint of pharmacokinetics and pharmacodynamics. In fact, pharmacokinetic factors significantly influence drug–receptor binding in physiological and pathological conditions. Thus, the *in vivo* and *ex vivo* characterization of drug–receptor binding may introduce a third dimension to preclinical pharmacokinetics and pharmacodynamics, with potential application not only in urology, but also in other therapeutic areas.

Competing Interests

There are no competing interests to declare.

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REFERENCES

- 1 Sarma AV, Jacobson DJ, McGree ME, Roberts RO, Lieber MM, Jacobsen SJ. A population based study of incidence and treatment of benign prostatic hyperplasia among residents of Olmsted County, Minnesota: 1987 to 1997. *J Urol* 2005; 173: 2048–53.
- 2 Glasser DB, Carson CI, Kang J, Laumann EO. Prevalence of storage and voiding symptoms among men aged 40 years and older in a US population-based study: results from the the Male Attitudes Regarding Sexual Health study. *Int J Clin Pract* 2007; 61: 1294–300.
- 3 Cheung WW, Khan NH, Choi KK, Bluth MH, Vincent MT. Prevalence, evaluation and management of overactive bladder in primary care. *BMC Fam Pract* 2009; 10: 8.

- 4 Townsend MK, Curhan GC, Resnick NM, Grodstein F. The incidence of urinary incontinence across Asian, black, and white women in the United States. *Am J Obstet Gynecol* 2010; 202: 378, e1–e7.
- 5 Kawabe K, Moriyama N, Yamada S, Taniguchi N. Rationale for the use of α -blockers in the treatment of benign prostatic hyperplasia (BPH). *Int J Urol* 1994; 1: 203–11.
- 6 Andersson KE. Alpha-adrenoceptors and benign prostatic hyperplasia: basic principles for treatment with alpha-adrenoceptor antagonists. *World J Urol* 2002; 19: 390–6.
- 7 Andersson KE. Muscarinic receptor antagonists for treatment of overactive bladder. *Lancet Neurol* 2004; 3: 46–53.
- 8 Ouslander JG. Management of overactive bladder. *N Engl J Med* 2004; 350: 786–99.
- 9 Honda K, Miyata-Osawa A, Takenaka T. Alpha 1-Adrenoceptor subtype mediating contraction of the smooth muscle in the lower urinary tract and prostate of rabbits. *Naunyn Schmiedebergs Arch Pharmacol* 1985; 330: 16–21.
- 10 Yamagishi R, Akiyama K, Nakamura S, Hora M, Masuda N, Matsuzawa A, Murata S, Ujiie A, Kurashina Y, Iizuka K, Kitazawa M. Effect of KMD-3213, an alpha 1a-adrenoceptor-selective antagonist, on the contractions of rabbit prostate and rabbit and rat aorta. *Eur J Pharmacol* 1996; 315: 73–9.
- 11 Suzuki Y, Kanada A, Okaya Y, Aisaka K, Muramatsu I. Effect of JTH-601, a novel alpha1-adrenoceptor antagonist, on the function of lower urinary tract and blood pressure. *Eur J Pharmacol* 1999; 374: 495–502.
- 12 Akiyama K, Hora M, Tatemichi S, Masuda N, Nakamura S, Yamagishi R, Kitazawa M. KMD-3213, a uroselective and long-acting alpha(1a)-adrenoceptor antagonist, tested in a novel rat model. *J Pharmacol Exp Ther* 1999; 291: 81–91.
- 13 Hieble JP, Bylund DB, Clarke DE, Eikenburg DC, Langer SZ, Lefkowitz RJ, Minneman KP, Ruffolo RR Jr. International Union of Pharmacology X. Recommendation for nomenclature of alpha 1-adrenoceptors: consensus update. *Pharmacol Rev* 1995; 47: 267–70.
- 14 Michel MC, Kenny B, Schwinn DA. Classification of alpha 1-adrenoceptor subtypes. *Naunyn Schmiedebergs Arch Pharmacol* 1995; 352: 1–10.
- 15 Michel MC, Vrydag W. α_1 -, α_2 - and β -adrenoceptors in the urinary bladder, urethra and prostate. *Br J Pharmacol* 2006; 147: S88–S119.
- 16 Farrall AJ, Wardlaw JM. Blood-brain-barrier: ageing and microvascular disease: systematic review and meta-analysis. *Neurobiol Aging* 2009; 30: 337–52.
- 17 Flicker C, Ferris SH, Serby M. Hypersensitivity to scopolamine in the elderly. *Psychopharmacology* 1992; 107: 437–41.
- 18 Molchan SE, Martinez RA, Hill JL, Weingartner HJ, Thompson K, Vitiello B, Sunderland T. Increased cognitive sensitivity to scopolamine with age and a perspective on the scopolamine model. *Brain Res Brain Res Rev* 1992; 17: 215–26.
- 19 Norbury R, Travis MJ, Erlandsson K, Waddington W, Owens J, Ell PJ, Murphy DG. SPET imaging of central muscarinic receptors with (R,R)[¹²³I]-QNB: methodological considerations. *Nucl Med Biol* 2004; 31: 583–90.
- 20 Yamada S, Suzuki M, Kato Y, Kimura R, Mori R, Matsumoto K, Maruyama M, Kawabe K. Binding characteristics of naftopidil and α_1 -adrenoceptor antagonists to prostatic a-adrenoceptors in benign prostatic hypertrophy. *Life Sci* 1992; 50: 127–35.
- 21 Yamada S, Tanaka C, Kimura R, Kawabe K. Alpha 1-adrenoceptors in human prostate: characterization and binding characteristics of alpha 1-antagonists. *Life Sci* 1994; 54: 1845–54.
- 22 Maruyama S, Oki T, Otsuka A, Shinbo H, Ozono S, Kageyama S, Mikami Y, Araki I, Takeda M, Masuyama K, Yamada S. Human muscarinic receptor binding characteristics of muscarinic receptor antagonists to treat overactive bladder. *J Urol* 2006; 175: 365–9.
- 23 Yoshida A, Fujino T, Maruyama S, Ito Y, Taki Y, Yamada S. Bladder selectivity based on *in vivo* drug-receptor binding characteristics of muscarinic receptor antagonists for treatment of overactive bladder. *J Pharmacol Sci* 2010; 112: 142–50.
- 24 Yazawa H, Takanashi M, Sudoh K, Inagaki O, Honda K. Characterization of [³H]YM617, R-(-)-5[2[[2]ethoxyring(n)-³H](o-ethoxyphenoxy)ethyl]amino]-propyl]-2-methoxybenzenesulfonamide HCl, a potent and selective alpha-1 adrenoceptor radioligand. *J Pharmacol Exp Ther* 1992; 263: 201–6.
- 25 Yamada S, Ohkura T, Kimura R, Kawabe K. *In vivo* receptor binding of novel α_1 -adrenoceptor antagonists for treatment of benign prostatic hyperplasia. *Life Sci* 1998; 62: 1585–9.
- 26 Ohkura T, Yamada S, Deguchi Y, Kimura R. Comparative study on pharmacokinetics and *in vivo* α_1 -adrenoceptor binding of [³H]tamsulosin and [³H]prazosin in rats. *Biol Pharm Bull* 1999; 22: 412–7.
- 27 Yamada S, Ohkura T, Deguchi Y, Kimura R. *In vivo* measurement by [³H]tamsulosin of α_1 -adrenoceptors in rat tissues in relation to the pharmacokinetics. *J Pharmacol Exp Ther* 1999; 289: 1575–83.
- 28 Yamada S, Okura T, Kimura R. *In vivo* demonstration of α_{1A} -adrenoceptor subtype selectivity of KMD-3213 in rat tissues. *J Pharmacol Exp Ther* 2001; 296: 160–7.
- 29 Martin DJ, Lluel P, Guillot E, Coste A, Jammes D, Angel I. Comparative alpha-1 adrenoceptor subtype selectivity and functional uroselectivity of alpha-1 adrenoceptor antagonists. *J Pharmacol Exp Ther* 1997; 282: 228–35.
- 30 Testa R, Guarneri L, Ibba M, Strada G, Poggessi E, Taddei C, Simonazzi I, Leonardi A. Characterization of alpha 1-adrenoceptor subtypes in prostate and prostatic urethra of rat, rabbit, dog and man. *Eur J Pharmacol* 1993; 249: 307–15.
- 31 Lepor H, Zhang W, Kobayashi S, Tang R, Wang B, Shapiro E. A comparison of the binding and functional properties of alpha-1 adrenoceptors and area density of smooth muscle in the human, canine and rat prostates. *J Pharmacol Exp Ther* 1994; 270: 722–7.

- 32 Shibata K, Foglar R, Horie K, Obika K, Sakamoto A, Ogawa S, Tsujimoto G. KMD-3213, a novel, potent, alpha 1a-adrenoceptor-selective antagonist: characterization using recombinant human alpha 1-adrenoceptors and native tissues. *Mol Pharmacol* 1995; 48: 250–8.
- 33 Michel MC, Büscher R, Kerker J, Kraneis H, Erdbrügger W, Brodde OE. Alpha 1-adrenoceptor subtype affinities of drugs for the treatment of prostatic hypertrophy. Evidence for heterogeneity of chloroethylclonidine-resistant rat renal alpha 1-adrenoceptor. *Naunyn Schmiedebergs Arch Pharmacol* 1993; 348: 385–95.
- 34 Ohkura T, Yamada S, Deguchi Y, Kimura R, Matsushima H, Higuchi S, Inagaki O, Honda K, Takenaka T. *Ex vivo* occupancy by tamsulosin of α_1 -adrenoceptors in rat tissues in relation to the plasma concentration. *Life Sci* 1998; 63: 2147–55.
- 35 Ohkura T, Yamada S, Abe Y, Kimura R. Selective and sustained occupancy of prostatic α_1 -adrenoceptors by oral administration of KMD-3213 and its plasma concentration in rats. *J Pharm Pharmacol* 2002; 54: 975–82.
- 36 Michel MC, Goepel M. Differential α_1 -adrenoceptor labeling by [3 H]prazosin and [3 H]tamsulosin. *Eur J Pharmacol* 1998; 342: 85–92.
- 37 Aboud R, Shafii M, Docherty JR. Investigation of the subtypes of alpha 1-adrenoceptor mediating contractions of rat aorta, vas deferens and spleen. *Br J Pharmacol* 1993; 109: 80–7.
- 38 Kenny BA, Chalmers DH, Philpott PC, Naylor AM. Characterization of an alpha 1D-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. *Br J Pharmacol* 1995; 115: 981–6.
- 39 Noble AJ, Chess-Williams R, Couldwell C, Furukawa K, Uchiyama T, Korstanje C, Chapple CR. The effects of tamsulosin, a high affinity antagonist at functional alpha 1A- and alpha 1D-adrenoceptor subtypes. *Br J Pharmacol* 1997; 120: 231–8.
- 40 Taguchi K, Saitoh M, Sato S, Asano M, Michel MC. Effects of tamsulosin metabolites at alpha-1 adrenoceptor subtypes. *J Pharmacol Exp Ther* 1997; 280: 1–5.
- 41 Murata S, Taniguchi T, Muramatsu I. Pharmacological analysis of the novel, selective alpha1-adrenoceptor antagonist, KMD-3213, and its suitability as a tritiated radioligand. *Br J Pharmacol* 1999; 127: 19–26.
- 42 Yarker YE, Goa KL, Fitton A. Oxybutynin. A review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic use in detrusor instability. *Drugs Aging* 1995; 6: 243–62.
- 43 Anderson RU, Mobley D, Blank B, Saltzstein D, Susset J, Brown JS. Once daily controlled versus immediate release oxybutynin chloride for urge urinary incontinence. OROS Oxybutynin Study Group. *J Urol* 1999; 161: 1809–12.
- 44 Davila GW, Daugherty CA, Sanders SW; Transdermal Oxybutynin Study Group. A short-term, multicenter, randomized double-blind dose titration study of the efficacy and anticholinergic side effects of transdermal compared to immediate release oral oxybutynin treatment of patients with urge urinary incontinence. *J Urol* 2001; 166: 140–5.
- 45 Dmochowski RR, Sand PK, Zinner NR, Gittelman MC, Davila GW, Sanders SW; Transdermal Oxybutynin Study Group. Comparative efficacy and safety of transdermal oxybutynin and oral tolterodine versus placebo in previously treated patients with urge and mixed urinary incontinence. *Urology* 2003; 62: 237–42.
- 46 Oki T, Toma-Okura A, Yamada S. Advantages for transdermal over oral oxybutynin to treat overactive bladder: muscarinic receptor binding, plasma drug concentration, and salivary secretion. *J Pharmacol Exp Ther* 2006; 316: 1137–45.
- 47 Waldeck K, Larsson B, Andersson KE. Comparison of oxybutynin and its active metabolite, N-desethyl-oxybutynin, in the human detrusor and parotid gland. *J Urol* 1997; 57: 1093–7.
- 48 Oki T, Kawashima A, Uchida M, Yamada S. *In vivo* demonstration of muscarinic receptor binding activity of N-desethyl-oxybutynin, active metabolite of oxybutynin. *Life Sci* 2005; 76: 2445–56.
- 49 Nilvebrant L, Andersson KE, Gillberg PG, Stahl M, Sparf B. Tolterodine – a new bladder-selective antimuscarinic agent. *Eur J Pharmacol* 1997; 327: 195–207.
- 50 Nilvebrant L, Gillberg PG, Sparf B. Antimuscarinic potency and bladder selectivity of PNU-200577, a major metabolite of tolterodine. *Pharmacol Toxicol* 1997; 81: 169–72.
- 51 Postlind H, Danielson A, Lindgren A, Andersson SH. Tolterodine, a new muscarinic receptor antagonist, is metabolized by cytochromes P450 2D6 and 3A in human liver microsomes. *Drug Metab Dispos* 1998; 26: 289–93.
- 52 Oki T, Sato S, Miyata K, Yamada S. Muscarinic receptor binding, plasma concentration and inhibition of salivation after oral administration of a novel antimuscarinic agent, solifenacin succinate in mice. *Br J Pharmacol* 2005; 145: 219–27.
- 53 Oki T, Maruyama S, Takagi Y, Yamamura HI, Yamada S. Characterization of muscarinic receptor binding and inhibition of salivation after oral administration of tolterodine in mice. *Eur J Pharmacol* 2006; 529: 157–63.
- 54 Maruyama S, Hasuike N, Suzuki K, Yamada S. *In vivo* characterisation of muscarinic receptors in peripheral tissues: evaluation of bladder selectivity of muscarinic receptor antagonists to treat overactive bladder. *Naunyn Schmiedebergs Arch Pharmacol* 2008; 377: 463–71.
- 55 Pählman I, d'Argy R, Nilvebrant L. Tissue distribution of tolterodine, a muscarinic receptor antagonist, and transfer into fetus and milk in mice. *Arzneimittelforschung* 2001; 51: 125–33.
- 56 Stöhrer M, Madersbacher H, Richter R, Wehnert J, Dreikorn K. Efficacy and safety of propiverine in SCI-patients suffering from detrusor hyperreflexia – a double-blind, placebo-controlled clinical trial. *Spinal Cord* 1999; 37: 196–200.
- 57 Madersbacher H, Halaska M, Voigt R, Alloussi S, Höfner K. A placebo-controlled, multicentre study comparing the tolerability and efficacy of propiverine and oxybutynin in patients with urgency and urge incontinence. *BJU Int* 1999; 84: 646–51.

- 58 Oki T, Yamada S, Toma A, Kimura R. Muscarinic receptor binding characteristics in rat tissues after oral administration of oxybutynin and propiverine. *Biol Pharm Bull* 2001; 24: 491–5.
- 59 Ito Y, Kato A, Fujino-Oki T, Okura T, Yoshida K, Nanri M, Yamada S. Muscarinic receptor binding and plasma drug concentration after the oral administration of propiverine in mice. *LUTS* 2010; 2: 43–9.
- 60 Yamada S, Ito Y, Taki Y, Seki M, Nanri M, Yamashita F, Morishita K, Komoto I, Yoshida K. The N-oxide metabolite contributes to bladder selectivity due to oral propiverine: muscarinic receptor binding and pharmacokinetics. *Drug Metab Dis* 2010; 38: 1314–21.
- 61 Kobayashi F, Yageta Y, Segawa M, Matsuzawa S. Effects of imidafenacin (KRP-197/ONO-8025), a new anti-cholinergic agent, on muscarinic acetylcholine receptors. High affinities for M₃ and M₁ receptor subtypes and selectivity for urinary bladder over salivary gland. *Arzneimittelforschung* 2007; 57: 92–100.
- 62 Kobayashi F, Yageta Y, Yamazaki T, Wakabayashi E, Inoue M, Segawa M, Matsuzawa S. Pharmacological effects of imidafenacin (KRP-197/ONO-8025), a new bladder selective anti-cholinergic agent, in rats. Comparison of effects on urinary bladder capacity and contraction, salivary secretion and performance in the Morris water maze task. *Arzneimittel forschung* 2007; 57: 147–54.
- 63 Homma Y, Yamaguchi O. Long-term safety, tolerability, and efficacy of the novel anti-muscarinic agent imidafenacin in Japanese patients with overactive bladder. *Int J Urol* 2008; 15: 986–91.
- 64 Homma Y, Yamaguchi O; Imidafenacin Study Group. A randomized, double-blind, placebo- and propiverine-controlled trial of the novel antimuscarinic agent imidafenacin in Japanese patients with overactive bladder. *Int J Urol* 2009; 16: 499–506.
- 65 Yamada S, Seki M, Ogoda M, Furuta A, Nakamura M, Ito Y. Selective binding of bladder muscarinic receptors in relation to the pharmacokinetics of a novel antimuscarinic agents, imidafenacin, to treat overactive bladder. *J Pharmacol Exp Ther* 2011; 336: 365–71.
- 66 Kornblum HI, Araujo DM, Annala AJ, Tatsukawa KJ, Phelps ME, Cherry SR. *In vivo* imaging of neuronal activation and plasticity in the rat brain by high resolution positron emission tomography (microPET). *Nat Biotech* 2000; 18: 655–60.
- 67 Mizuta T, Kitamura K, Iwata H, Yamagishi Y, Ohtani A, Tanaka K, Inoue Y. Performance evaluation of a high-sensitivity large-aperture small-animal PET scanner: ClairvivoPET. *Ann Nucl Med* 2008; 22: 447–55.
- 68 Tsukada H. Delivery of radioligands for positron emission tomography (PET) in the central nervous system. *Adv Drug Deliv Rev* 1999; 37: 175–88.
- 69 Halldin C, Gulyas B, Farde L. PET studies with carbon-11 radioligands in neuropsychopharmacological drug development. *Curr Pharm Des* 2001; 7: 1907–29.
- 70 Yoshida Y, Maruyama S, Fukumoto D, Tsukada H, Ito Y, Yamada S. Noninvasive evaluation of brain muscarinic receptor occupancy of oxybutynin, darifenacin and imidafenacin in rats by positron emission tomography. *Life Sci* 2010; 25: 175–80.
- 71 Katz IR, Sands LP, Bilker W, DiFilippo S, Boyce A, D'Angelo K. Identification of medications that cause cognitive impairment in older people: the case of oxybutynin chloride. *J Am Geriatr Soc* 1998; 46: 8–13.
- 72 Ancelin ML, Artero S, Portet F, Dupuy AM, Touchon J, Ritchie K. Non-degenerative mild cognitive impairment in elderly people and use of anticholinergic drugs: longitudinal cohort study. *BMJ* 2006; 332: 455–59.
- 73 Maruyama S, Tsukada H, Nishiyama S, Kakiuchi T, Fukumoto D, Oku N, Yamada S. *In vivo* quantitative autoradiographic analysis of brain muscarinic receptor occupancy by muscarinic receptor antagonists for overactive bladder treatment. *J Pharmacol Exp Ther* 2008; 325: 774–81.
- 74 Oki T, Kageyama A, Takagi Y, Uchida S, Yamada S. Comparative evaluation of central muscarinic receptor binding activity by oxybutynin, tolterodine and darifenacin used to treat overactive bladder. *J Urol* 2007; 177: 766–70.
- 75 Kay G, Crook T, Rebeda L, Lima R, Ebinger U, Arguinzoniz M, Steel M. Differential effects of the muscarinic receptor antagonists darifenacin and oxybutynin ER on memory in older subjects. *Eur Urol* 2006; 50: 317–26.
- 76 Suzuki M, Noguchi Y, Okutsu H, Ohtake A, Sasamata M. Effect of antimuscarinic drugs used for overactive bladder on learning in a rat passive avoidance response test. *Eur J Pharmacol* 2007; 557: 154–58.
- 77 Scheife R, Takeda M. Central nervous system safety of anticholinergic drugs for the treatment of overactive bladder in the elderly. *Clin Ther* 2005; 27: 144–53.
- 78 Clemett D, Jarvis B. Tolterodine: a review of its use in the treatment of overactive bladder. *Drugs Aging* 2001; 18: 277–304.
- 79 Skerjanec A. The clinical pharmacokinetics of darifenacin. *Clin Pharmacokinet* 2006; 45: 325–50.
- 80 Oki T, Takagi Y, Inagaki S, Taketo MM, Manabe T, Matsui M, Yamada S. Quantitative analysis of binding parameters of [³H]N-methylscopolamine in central nervous system of muscarinic acetylcholine receptor knockout mice. *Brain Res Mol Brain Res* 2005; 133: 6–11.
- 81 Kay GG, Abou-Donia MB, Messer WS Jr, Murphy DG, Tsao JW, Ouslander JG. Antimuscarinic drugs for overactive bladder and their potential effects on cognitive function in older patients. *J Am Geriatr Soc* 2005; 53: 2195–201.
- 82 Yamada S, Maruyama S, Takagi Y, Uchida S, Oki T. *In vivo* demonstration of M₃ muscarinic receptor subtype selectivity of darifenacin in mice. *Life Sci* 2006; 80: 127–32.
- 83 Ito Y, Oyuncul L, Yoshida A, Fujino T, Noguchi Y, Yuyama H, Ohtake A, Suzuki M, Sasamata M, Matsui M, Yamada S. Comparison of muscarinic selectivity of solifenacin and oxybutynin in the bladder and submandibular gland of muscarinic receptor knockout mice. *Eur J Pharmacol* 2009; 615: 201–6.

- 84** Birder LA, Ruan HZ, Chopra B, Xiang Z, Barrick S, Buffington CA, Roppolo JR, Ford AP, de Groat WC, Burnstock G. Alterations in P2X and P2Y purinergic receptor expression in urinary bladder from normal cats and cats with interstitial cystitis. *Am J Physiol Renal Physiol* 2004; 287: F1084–91.
- 85** Giglio D, Ryberg AT, To K, Delbro DS, Tobin G. Altered muscarinic receptor subtype expression and functional responses in cyclophosphamide induced cystitis in rats. *Auton Neurosci* 2005; 122: 9–20.
- 86** Kageyama A, Fujino T, Taki Y, Kato Y, Nozawa Y, Ito Y, Yamada S. Alteration of muscarinic and purinergic receptors in urinary bladder of rats with cyclophosphamide-induced interstitial cystitis. *Neurosci Lett* 2008; 436: 81–4.
- 87** Yoshida A, Kageyama A, Fujino T, Nozawa Y, Yamada S. Loss of muscarinic and purinergic receptors in the urinary bladder of rats with hydrochloric acid-induced cystitis. *Urol* 2010; 76: 1017, e7–e12.
- 88** Ito Y, Oyunzul L, Seki M, Fujino-Okai T, Matsui M, Yamada S. Quantitative analysis of the loss of muscarinic receptors in various peripheral tissues in M₁-M₅ single subtype knockout mice. *Br J Pharmacol* 2009; 156: 1147–53.