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REVIEW

# Muscarinic receptors: their distribution and function in body systems, and the implications for treating overactive bladder

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**1** The effectiveness of antimuscarinic agents in the treatment of the overactive bladder (OAB) syndrome is thought to arise through blockade of bladder muscarinic receptors located on detrusor smooth muscle cells, as well as on nondetrusor structures.

**2** Muscarinic $M_3$ receptors are primarily responsible for detrusor contraction. Limited evidence exists to suggest that $M_2$ receptors may have a role in mediating indirect contractions and/or inhibition of detrusor relaxation. In addition, there is evidence that muscarinic receptors located in the urothelium/suburothelium and on afferent nerves may contribute to the pathophysiology of OAB. Blockade of these receptors may also contribute to the clinical efficacy of antimuscarinic agents.

**3** Although the role of muscarinic receptors in the bladder, other than $M_3$ receptors, remains unclear, their role in other body systems is becoming increasingly well established, with emerging evidence supporting a wide range of diverse functions. Blockade of these functions by muscarinic receptor antagonists can lead to similarly diverse adverse effects associated with antimuscarinic treatment, with the range of effects observed varying according to the different receptor subtypes affected.

**4** This review explores the evolving understanding of muscarinic receptor functions throughout the body, with particular focus on the bladder, gastrointestinal tract, eye, heart, brain and salivary glands, and the implications for drugs used to treat OAB. The key factors that might determine the ideal antimuscarinic drug for treatment of OAB are also discussed. Further research is needed to show whether the $M_3$ selective receptor antagonists have any advantage over less selective drugs, in leading to fewer adverse events.

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**Keywords:** Overactive bladder; muscarinic receptors; antimuscarinics; selective $M_3$ antagonists; bladder; salivary glands; gastrointestinal tract; brain; eye; heart

**Abbreviations:** ACh, acetylcholine; ATP, adenosine triphosphate; BBB, blood–brain barrier; CIBIC, clinician's interview based impression of change; CNS, central nervous system; CR, controlled release; DO, detrusor overactivity; ER, extended release; IR, immediate release; OAB, overactive bladder; $M_1$–$M_5$, muscarinic receptor subtypes 1–5; NK, neurokinin; 4-DAMP, 4-diphenylacetoxy-*N*-methylpiperidine; 5-HT, 5-hydroxytryptamine (serotonin)

## Introduction

Antimuscarinic agents are commonly used to treat patients suffering from the overactive bladder (OAB) syndrome (see Andersson *et al*., 2002; 2005). OAB is defined as urgency, with or without urgency incontinence, usually with increased daytime frequency and nocturia (Abrams *et al*., 2002). Antimuscarinic drugs have, for a long time, been considered to produce their beneficial effects by acting solely *via* muscarinic receptors located on detrusor smooth muscle. However, new evidence has led to the suggestion that antimuscarinics could work by affecting muscarinic receptors within the urothelium and on bladder afferent (sensory) nerves (see Andersson & Yoshida, 2003; Andersson, 2004).

## Distribution and functional role of muscarinic receptors

Muscarinic receptors are widely distributed throughout the human body and mediate distinct physiological functions according to location and receptor subtype (see Caulfield & Birdsall, 1998). Five distinct muscarinic receptor subtypes ($M_1$–$M_5$) are known to exist, although the exact location and functional role of all these subtypes has to date not been fully

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elucidated. In particular, these receptors may have differing but vital roles within the same body system, with potential interplay between subtypes. Thus, a thorough understanding of these differing muscarinic receptor subtypes is important.

## *The bladder*

Under normal conditions, human detrusor contractility is predominantly under the control of the parasympathetic nervous system, where the primary input is *via* acetylcholine (ACh) acting on muscarinic receptors.

Studies show that the detrusor muscle of various species (including humans) contains all muscarinic receptor subtypes but that $M_2$ and $M_3$ receptors are predominant, with the $M_2$ subtype outnumbering the $M_3$ receptor subtype (3 : 1 ratio) (see Wang *et al*., 1995; Hegde & Eglen, 1999). However, it is the minority population of $M_3$ receptors that mediate human detrusor contraction *in vitro* (Chess-Williams *et al*., 2001; Fetscher *et al*., 2002) (Figure 1), given that the correlation between functional affinity in human isolated detrusor and recombinant receptor affinity across a range of muscarinic antagonists is greatest for the $M_3$ subtype. Further evidence to support the functional role of the $M_3$ subtype comes from studies in $M_3$ knockout mice. In bladder strips from such mice, 95% of the contraction induced by carbachol is mediated by $M_3$ receptors, as shown by a reduction in the maximal contractile response to only 5% of that seen in wild-type mice (Matsui *et al*., 2000). However, these mice have an almost normal cystometric pattern owing to the remaining purinergic activation mechanism (Igawa *et al*., 2004).

The functional role of the large $M_2$ receptor population in detrusor muscle remains unclear. An investigation using $M_2$, $M_3$ and $M_2$/$M_3$ double knockout mice revealed that that the $M_2$ receptor may have a role in indirectly mediating bladder contractions by enhancing the contractile response to $M_3$ receptor activation, and that minor $M_2$ receptor-mediated contractions may also occur (Ehlert *et al*., 2005). The authors of another rodent study suggest that the stimulation of $M_2$ receptors may serve to inhibit sympathetically (i.e. beta-adrenoceptor) mediated relaxation, which in turn leads to more efficient emptying of the bladder (Hegde *et al*., 1997). A functional role for $M_2$ receptors in bladder function may emerge in certain disease states, as observed in studies of outflow obstruction in rats (Braverman *et al*., 1998; Braverman & Ruggieri, 2003) and neurogenic human bladder (Pontari *et al*., 2004). In denervated rat bladder, for example, there is an increase in $M_2$ receptor density (with a corresponding increase in the $M_2$ : $M_3$ ratio), with functional affinity of muscarinic antagonists more closely resembling their affinity for $M_2$ than for $M_3$ receptors (Braverman *et al*., 1998). However, the functional affinity of the $M_3$ selective antagonist 4-DAMP did not differ in normal and obstructed rat bladder (Krichevsky *et al*., 1999). Sympathetic modulation of the human bladder *via* $M_2$ receptors may also be inferred as noradrenergic innervation, albeit scarce, has been demonstrated in human bladder body and increases in the outflow region (see Gosling *et al*., 1999).

Two studies presented at the American Urological Association meeting in 2004 reported that the $M_3$ receptor was responsible for mediating the direct contractile response in human detrusor muscle tissue taken from patients with neurogenic and idiopathic detrusor overactivity (DO) and those with normal bladder function (Stevens *et al*., 2004a, b). Furthermore, no changes in receptor subtype contribution to detrusor contractions in the disease state were observed. The concentration–response curves to carbachol indicated that muscarinic receptor-mediated function was enhanced in the neurogenic and idiopathic DO tissue compared with normal bladder tissue *in vitro*. The presence of the $M_3$ receptor selective antagonist 4-DAMP reduced the contractile response to carbachol in the normal bladder and in the neurogenic and idiopathic DO, whereas the $M_2$ receptor selective antagonist, methoctramine, was less effective in all tissues. However, the study did not show any significant differences from unity in the Schild slopes for either antagonist (Stevens *et al*., 2004b). As such, an indirect role of $M_2$ receptors in mediating contractile responses cannot be discounted.

Findings from *in vitro* research using human and guinea-pig bladder tissue have led to the proposal that a network of interstitial cells – similar to the interstitial cells of Cajal in the gut (myofibroblasts) – within the suburothelial layer may augment and coordinate autonomous detrusor activity (see Fry *et al*., 2004; Gillespie, 2004a, b). Immunocytochemical evidence from rodents has also demonstrated the presence of $M_3$ receptors located on interstitial cells in the suburothelial layer (Gillespie *et al*., 2003), and it has been postulated that such cells expressing $M_3$ receptors may contribute to the generation of phasic contractions (Gillespie, 2004a, b), and may be activated by ACh generated and released from the urothelium/suburothelium (see Yoshida *et al*., 2004). One hypothesis is that such cells can activate phasic activity, whereas ATP-releasing and peptidergic neurons present within

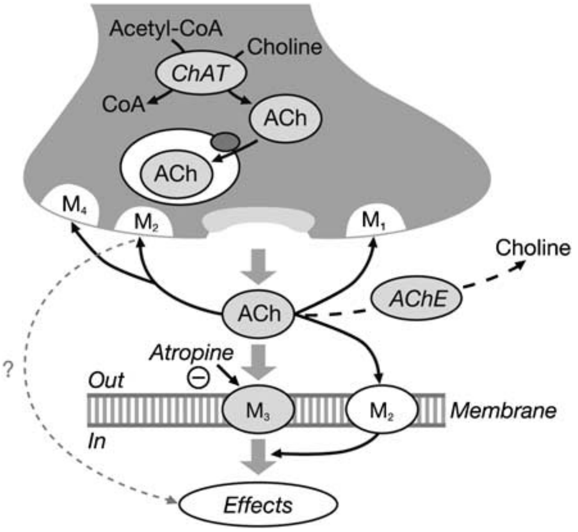

**Figure 1** The role of the M3 receptor in detrusor contraction. Acetylcholine (ACh), produced in the presynaptic terminal by the action of choline acetyl transferase (ChAT) on choline and acetyl coenzyme A (acetyl-CoA), is released by exocytosis. ACh is metabolized by acetyl cholinesterase (AChE) to release choline. Detrusor contraction is mediated by the binding of ACh on postjunctional membrane muscarinic $M_3$ receptors ($M_3$), resulting in activation of the contractile proteins within the detrusor muscle (Effects). Prejunctional $M_2$ and $M_4$ receptors inhibit, whereas prejunctional $M_1$ receptors facilitate the release of ACh. The $M_2$ receptor also appears to have an indirect functional role in detrusor contractility, and possibly a minor direct effect, but the mechanism remains unclear. Atropine inhibits contraction by blockade of muscarinic receptors.

the network of interstitial cells modulate bladder sensations. Indeed, activation of cholinergic receptors in feline epithelial cells has been shown to facilitate ATP release (Birder *et al*., 2003), which in turn may activate adjacent afferent nerves or myofibroblasts. Thus, inappropriate phasic activity could contribute to DO and be responsible for generating 'sensory urgency'. Although intriguing, further investigations are needed to understand the subtypes and functional role of muscarinic receptors within the urothelium.

Muscarinic receptors are also located prejunctionally on cholinergic nerve terminals within the bladder, where $M_1$ receptors facilitate transmitter release and $M_2/M_4$ receptors inhibit transmitter release (see Chess-Williams, 2002; Zhou *et al*., 2002). However, the functional role of these prejunctional receptors remains unclear (see Somogyi *et al*., 1996; Chess-Williams, 2002). Current *in vitro* research suggests that the $M_1$ receptor is a prominent modulator of ACh release, the stimulation of which, during increased nerve traffic, may act to promote more efficient voiding. Evidence also suggests that the prejunctional facilitatory receptors exhibit plasticity following spinal cord injury (see Somogyi & De Groat, 1999). Prejunctional high-affinity $M_3$ receptors at cholinergic nerve endings are upregulated in bladders of chronic spinal cord transected rats and replace low-affinity $M_1$ muscarinic receptors (Somogyi *et al*., 2003). Conversely, it has been suggested that inhibitory $M_2$ and $M_4$ prejunctional receptors may function to promote urine storage, with enhanced activity at times of low-frequency nerve traffic, for example in pathologic states such as bladder denervation or spinal cord injury (see Chapple, 2000).

It is clear that the control of normal and pathological bladder function and the functional role of muscarinic receptors is highly complex. It remains unknown as to whether the efficacy of antimuscarinic agents in the treatment of OAB is specific to an effect on $M_3$ receptors within the detrusor muscle, or whether actions at other receptor sites such as sensory nerves or urothelium/suburothelium contribute to the therapeutic effect.

## *The salivary glands*

The parasympathetic nervous system plays a pivotal role in the production of saliva by serous and mucous cells of the acinar structures in salivary glands (see Baum, 1993) and by serous cells in the parotid glands. Human and rodent studies show that both $M_1$ and $M_3$ receptors are present in the salivary glands, whereas the parotid glands express predominantly $M_3$ receptors (Culp *et al*., 1996; Watson *et al*., 1996; Beroukas *et al*., 2002). Similarly, functional studies in mice and rats have demonstrated that submandibular gland secretion is mediated through $M_1$ and $M_3$ receptors, whereas parotid gland secretion is mediated *via* $M_3$ (and possibly $M_4$) receptors (Tobin *et al*., 2002; Bymaster *et al*., 2003; Gautam *et al*., 2004); the robustness of these findings may be inferred from the finding that these effects were observed across different modes of induction of salivation (*via* electrical stimulation of the parasympathetic nervous system or stimulated by oxotremorine or pilocarpine). Thus, salivation is predominantly mediated by the $M_3$ receptors that are involved in the control of both high- and low-viscosity secretions and saliva volume, whereas the $M_1$ subtype is involved in the control of high-viscosity lubrication. This has been illustrated by preclinical studies in rats and cats which demonstrated that selective antagonism of $M_3$ receptors inhibits, but does not eliminate, salivary responses to carbachol or electrical stimulation (Gillberg *et al*., 1998; Ikeda *et al*., 2002).

Although salivation is primarily mediated by $M_3$ receptors, the functional importance of multiple muscarinic receptor subtypes in the quantity and quality of salivary secretion is highlighted by the fact that agonist-induced salivation (using oxotremorine, pilocarpine or isoproterenol) is depressed in the $M_3$ knockout mouse, yet the buccal cavity remains lubricated (Matsui *et al*., 2000; Bymaster *et al*., 2003). In contrast, mice devoid of $M_1$ and $M_4$ receptors show an intermediate response, whereas while $M_2$ and $M_5$ knockout mice have normal salivation (Bymaster *et al*., 2003). As pilocarpine-induced salivation is abrogated in $M_1/M_3$ receptor double-knockout mice (Gautam *et al*., 2004), and maximal salivary secretion induced by carbachol requires both $M_1$ and $M_3$ receptors (Luo *et al*., 2001), it is evident that salivation is mediated by two different postjunctional muscarinic receptors. In addition to the postjunctional receptors, there are neuronal $M_2$ and $M_1$ receptors on the nerves supplying the salivary glands. These neuronal receptors have a contributory role in salivation by inhibiting ($M_2$) or enhancing ($M_1$) ACh release from the nerves (Tobin, 2002).

In the clinical context, some studies have shown that $M_3$-selective and nonselective muscarinic receptor antagonists (with activity at both $M_1$ and $M_3$ receptors) appear to reduce salivation in similar proportions of patients (Diokno *et al*., 2003; Haab *et al*., 2004; Armstrong *et al*., 2005). In contrast, in a crossover study of 65 patients with OAB comparing darifenacin with oxybutynin, treatment with oxybutynin immediate release (IR) 5 mg three times daily was associated with significantly greater reductions ($P < 0.05$) in salivary flow than darifenacin controlled release (CR) (15 mg once daily or 30 mg once daily) (Chapple & Abrams, 2005). It is possible that, compared with antagonism of both receptor subtypes, sparing the $M_1$ receptors in the salivary glands may help to maintain enough lubrication to alleviate the sensation and severity of dry mouth. This is supported by low discontinuation rates owing to dry mouth (<3%) during darifenacin treatment, based on a pooled analysis of three darifenacin studies (Chapple *et al*., 2005).

## *The gastrointestinal tract*

Although gut smooth muscle has been shown to contain all five muscarinic receptor subtypes in differing proportions in guinea-pigs (So *et al*., 2003), $M_2$ and $M_3$ receptors are thought to be the most functionally important in humans. As with the bladder, $M_2$ receptors outnumber the $M_3$ receptor population by up to 4 : 1 in humans (Gomez *et al*., 1992; Kerr *et al*., 1995) but data from studies in rodents and dogs suggest that the $M_3$ receptor appears to play the prominent role in cholinergic stimulation of gastrointestinal motility (Eglen & Harris, 1993; Chiba *et al*., 2002; Li *et al*., 2002; Matsui *et al*., 2002). As such, antagonism of these receptors may contribute to a reduction in colonic transit. A functional role for other muscarinic receptor subtypes, particularly the $M_2$ receptor, is beginning to emerge (see Matsui *et al*., 2000; Eglen, 2001). Indeed, *in vitro* research using murine smooth muscle has indicated that $M_2$ receptors may have a greater contribution to contractility in the gastrointestinal tract than in the bladder (Matsui *et al*., 2000).

Numerous other signaling mechanisms, mediated by a variety of neurotransmitters within the enteric nervous system, also appear to play a major role in physiological control of gastrointestinal function. Serotonergic (5-HT) receptors have been shown to be important in the control of gastrointestinal motility and sensitivity. For example, the 5-$HT_4$ receptor subtype mediates excitatory effects (Gershon, 2003) and directly influences gastrointestinal secretion. Other signalling mechanisms implicated in the control of gastrointestinal function include substance P and neurokinin (NK) A acting at $NK_1$ and $NK_2$ receptors, and the inhibition of nitric oxide release. The complex interplay between these mechanisms helps explain why $M_3$ knockout mice have no overt gastrointestinal problems (Matsui *et al*., 2000).

As with the bladder, many gaps in knowledge still exist regarding the functional role of muscarinic receptors and the contribution of specific subtypes within the gastrointestinal tract. These include the role of muscarinic receptors expressed by interstitial cells of Cajal and enteric neurons, the role of $M_4$ and $M_5$ receptors on smooth muscle and the mechanisms of long-term compensation for muscarinic deprivation. In the clinical setting, constipation following muscarinic antagonist therapy is often reported as one of the classic muscarinic adverse events. This is to be expected given the need to target the $M_3$ receptor to achieve clinical efficacy in OAB, and the role of this receptor in the complex mechanisms involved in gastrointestinal transit. In a pooled analysis of fixed dose clinical studies with the $M_3$ selective receptor antagonist darifenacin, an increase was observed in the reported incidence of constipation compared with placebo (14.8 and 21.3% all-causality incidence for darifenacin CR 7.5 and 15 mg once daily, respectively, compared with 6.2% for placebo) (Chapple *et al*., 2005). Although the incidence of constipation appears to be higher with darifenacin than for other antimuscarinics, a clinical comparison of darifenacin and the nonselective muscarinic receptor antagonist tolterodine IR showed that that the two agents were associated with similar incidences of new-onset laxative use for constipation and discontinuations owing to constipation (Thomas *et al*., 2005). However, further detailed studies are needed to investigate the comparative clinical effects of $M_3$ selective and nonselective muscarinic receptor antagonists on the gastrointestinal tract.

## *The brain*

Muscarinic receptors in the brain activate a multitude of signaling pathways important for the modulation of neuronal excitability, synaptic plasticity and feedback regulation of ACh release. All five muscarinic receptor subtypes are expressed in the brain (see Volpicelli & Levey, 2004). $M_1$ receptors, for example, are most abundant in the neocortex, hippocampus and neostriatum, whereas $M_2$ receptors are located throughout the brain. In contrast, levels of $M_3$ receptors are low whereas $M_4$ receptors are abundant in the neostriatum, and $M_5$ receptors have been localized to the projection neurons of substantia nigra, pars compacta, ventral tegmental area and the hippocampus (Table 1).

**Table 1** Location of muscarinic receptors in the brain

*$M_1$: Abundant in neocortex, hippocampus and neostriatum*
Pyramidal cells
Small fraction appear to be on axons and terminals

*$M_2$: Throughout brain*
Autoreceptor (inhibitory) in hippocampus and cortex
Noncholinergic terminals in hippocampus, cortex and olfactory bulb
Basal forebrain (e.g. GABAergic neurons in visual cortex)
Thalamus

*$M_3$: Low levels throughout brain*
Hippocampus
Thalamus
Striatal GABAergic neurons

*$M_4$: Abundant in neostriatum; also in the cortex and hippocampus*
Autoreceptor (inhibitory) in striatum
Striatal medium spiny neurons
Hippocampus

*$M_5$: Projection neurons of the substantia nigra, pars compacta and ventral tegmental area; also in this hippocampus*
Dopaminergic terminals stimulatory in the nucleus accumbens and striatum

GABA, gamma-aminobutyric acid.

Central muscarinic receptors are involved in higher cognitive processes such as learning and memory. It is generally accepted that $M_1$ receptors play an important functional role in this regard. Indeed, antagonism of central $M_1$ receptors with intrahippocampal pirenzepine impaired spatial memory in rat models (Messer *et al*., 1990). Also, mice lacking the $M_1$ receptor exhibit defects in a number of cognitive processes (Anagnostaras *et al*., 2003), and $M_1$ receptor agonists reverse learning and spatial memory impairment in animal models of Alzheimer's disease (see Fisher *et al*., 2003). In clinical studies, an $M_1/M_4$ receptor agonist has been reported to improve cognition in patients with Alzheimer's disease, as measured on the Clinician's Interview Based Impression of Change, although treatment was associated with a high incidence of systemic side effects (Bodick *et al*., 1997). Central $M_1$ antagonism may therefore give rise to cognitive dysfunction and other central nervous system (CNS)-related adverse events. These effects are becoming increasingly associated with antimuscarinic agents with a relatively high affinity for this receptor (Donnellan *et al*., 1997; Katz *et al*., 1998; Womack & Heilman, 2003). Moreover, activity at central $M_2$ receptors may also contribute to impaired cognitive function, given that mice devoid of such receptors display cognitive deficits (Tzavara *et al*., 2003). These findings were expanded in a further study, which showed a deficit in behavioral flexibility, working memory and hippocampal plasticity in $M_2$ knockout mice (Seeger *et al*., 2004). Although such studies cannot be replicated in man, Perry *et al*. (2003) showed that patients with Parkinson's disease, treated for over 2 years with less selective antimuscarinic agents (orphenadrine, oxybutynin, trihexyphenidyl), had more pathological stigmata of Alzheimer's disease than patients who had not been treated with these agents. These findings suggest that both $M_1$ and $M_2$ receptors in the CNS play an important functional role in cognitive function. In contrast, $M_3$ knockout mice show normal cognition and behavior (Yamada *et al*., 2001a).

It is also important to note that antagonism of muscarinic $M_1$ and $M_2$ receptors in the brain is dependent not only on a drug's affinity for these receptors, but also on the drug concentration within the CNS. This is determined by the balance between drug penetration through the blood–brain barrier (BBB) and efflux. Thus, the molecular size, polarity and lipophilicity, and specificity for the P-glycoprotein efflux pump may influence the risk of adverse CNS effects with antimuscarinic drugs. However, the drug levels in the CNS may change in situations where the BBB becomes 'leaky' following damage (e.g. under conditions of stress, advanced age or presence of comorbid conditions such as diabetes or multiple sclerosis) (see Liebsch *et al*., 1996; Habgood *et al*., 2000; Pakulski *et al*., 2000; Esposito *et al*., 2001; Starr *et al*., 2003; Ballabh *et al*., 2004). Indeed, older individuals, who are often prescribed multiple medications that have antimuscarinic activity (see Tune, 2001), are particularly susceptible to their CNS adverse effects, with likely contributory factors including age-related changes in drug elimination, increased BBB permeability and reductions in muscarinic receptor density. It is notable that in clinical trials, a low incidence of CNS changes and CNS adverse events has been reported with oxybutynin extended release (ER) and tolterodine ER, and these events were rarely a cause for discontinuation (see Clemett & Jarvis, 2001; Chu *et al*., 2005). However, significant effects on quantitative electroencephalogram, sleep physiology and cognitive test performance have been demonstrated with nonselective antimuscarinic therapy (Pietzko *et al*., 1994; Katz *et al*., 1998; Diefenbach *et al*., 2003). In contrast, emerging evidence suggests that $M_1$/$M_2$ receptor sparing antimuscarinic therapy may be free of CNS sedation and cognitive impairment, although it should be noted that these studies did not employ a nonselective OAB antimuscarinic as a comparator (Kay & Wesnes, 2005; Lipton *et al*., 2005).

Mechanisms implicated in increased BBB permeability include epithelial shrinkage accompanied by opening of tight junctions and dilation of the blood vessels resulting in increased blood flow and enhanced transport, as shown in a rat model (Abdel-Rahman *et al*., 2002). Other mechanisms could include enhanced pinocytotic activity, which is seen with increasing age (Pakulski *et al*., 2000). Consequently, all antimuscarinic receptor antagonists, irrespective of their physicochemical properties, have the potential to cross the BBB, although the level of affinity/serum concentration needed to affect muscarinic receptors mediating cognitive function requires investigation. However, available evidence suggests that a key issue regarding the potential for minimizing any cognitive adverse events with antimuscarinic agents would be to spare the $M_1$ receptor.

### *The eye*

The findings of immunoprecipitation studies show that all five muscarinic receptor subtypes exist within the human eye, of which the $M_3$ receptor predominates ($M_3$, 60–75%; $M_2$, 5–10%; $M_4$, 5–10%; $M_1$, 7%, in ciliary processes and iris sphincter; $M_5$, 5%, located only in the iris sphincter) (Gil *et al*., 1997; Ishizaka *et al*., 1998). Functional $M_3$ receptors mediating contractility responses have been identified on trabecular meshwork, ciliary muscle and iris sphincter of cows (Wiederholt *et al*., 1996), Rhesus monkeys (Poyer *et al*., 1994) and humans, respectively (Woldemussie *et al*., 1993; Shade *et al*., 1996). Functional studies in $M_3$ knockout mice have lent support to the involvement of $M_3$ receptors in controlling iris sphincter contraction (Matsui *et al*., 2000; Bymaster *et al*., 2003) with other studies in the canine or rabbit eye suggesting that $M_5$ receptors also contribute to cholinergically mediated contraction of isolated ciliary muscle (Bognar *et al*., 1992; Choppin & Eglen, 2001). Studies using knockout mice have suggested that $M_2$ receptors may be involved in additional mechanisms controlling pupillary constriction and dilation. Mice lacking both $M_2$ and $M_3$ receptors had pupil constriction compared with mice lacking the $M_3$ receptor only (Matsui *et al*., 2002). Whether this observation reflects an effect mediated through autoreceptors was speculated, but is currently unknown. Other studies have suggested that $M_2$ receptors on parasympathetic and sympathetic nerve terminals in the iris can modulate ACh release in rabbits and and norepinephrine release in humans, respectively (Bognar *et al*., 1990; Jumblatt & Hackmiller, 1994). A role for $M_2$ receptors in the regulation of trabecular meshwork contractility has also been suggested from studies examining human and bovine tissue (Thieme *et al*., 2001).

Although $M_1$ and $M_4$ receptors are expressed in the human eye (Gil *et al*., 1997; Ishizaka *et al*., 1998), their functional roles have yet to be fully elucidated. $M_1$ receptors are present in human iris, sclera and native lens epithelial cells, where they are the predominant subtype and mediate changes in cytosolic calcium (Collison *et al*., 2000). A functional role for $M_4$ receptors in the eye remains to be determined. Of note, animal studies have shown that $M_1$, $M_2$ and $M_3$ receptors can mediate activation of conjunctival goblet cells – the primary source of mucins in the tear film (Kanno *et al*., 2003).

The propensity for an antimuscarinic agent to cause ocular events will depend upon a number of factors. Consideration should be given to the serum levels necessary to affect structures within the eye, and the specific affinities of the muscarinic receptors present with a given serum level of drug. As such, although ocular events may be seen with both $M_3$ and $M_5$ receptor antagonism, blurred vision is uncommon with the selective $M_3$ receptor antagonist darifenacin, with one comparative study reporting no episodes of blurred vision in contrast to a 3% rate with the less selective agent oxybutynin (Zinner *et al*., 2005).

Similar to the brain, the potential for adverse effects in the eye with a particular antimuscarinic may not only depend on the selectivity of the drug but also its physical characteristics, potential to cross the blood–retina barrier, which regulates permeation of substances from the blood to the retina (see Duvvuri *et al*., 2003), and affinity for potential mechanisms regulating efflux.

### *The heart*

Stimulation of muscarinic receptors within the mammalian heart, specifically the $M_2$ subtype (see Hulme *et al*., 1990; Caulfield, 1993), modulates pacemaker activity and atrioventricular conduction, and directly (in atrium) or indirectly (in ventricles) the force of contraction (see Dhein *et al*., 2001). Indeed, bradycardia in response to carbachol is abolished in $M_2$ knockout mice (Stengel *et al*., 2000), emphasizing the functional importance of this subtype. Although other muscarinic receptor subtypes have also been localized in the human heart ($M_1$, $M_3$ and $M_5$) (Hellgren *et al*., 2000; Wang *et al*., 2001; Willmy-Matthes *et al*., 2003), details of their functional roles are still emerging.

Functional $M_1$ receptors, which increase heart rate, have been reported in rodent cardiac tissue (Islam *et al*., 1998;

Colecraft *et al*., 1998) and human atrial myocytes (Dobrev *et al*., 2003; Wang *et al*., 2004). However, the basal heart function of mice lacking $M_1$ receptors is unchanged compared with wild type, and cardiac stimulation by $M_1$ receptors occurs through stimulation of catecholamine release from sympathetic neurons (Hardouin *et al*., 2002). Other studies have also implicated non-$M_2$ receptors, in addition to $M_2$ receptors, in the modulation of sympathetic neurotransmitter release in mouse atria (Trendelenburg *et al*., 2003).

Functional $M_3$ receptors have been identified in rodent and mammalian cardiac tissue (see Nishimaru *et al*., 2000; Pönicke *et al*., 2003; Wang *et al*., 2004) and in human atrial tissue (Dobrev *et al*., 2003; Willmy-Matthes *et al*., 2003), although data obtained using knockout mice suggest limited involvement of $M_3$ receptors in physiological cardiac function. Studies using mice lacking either $M_2$ or $M_3$ receptors have indicated an obligatory role for $M_2$ receptors in heart-rate regulation, and no change in the basal heart rate of $M_3$ knockout mice (Gomeza *et al*., 1999; Stengel *et al*., 2002).

It is important to consider whether the role of muscarinic receptor subtypes in modulating cardiac function may alter in pathological conditions. Additional data have indicated increased $M_3$ receptor density, but a decrease in $M_2$ receptors, in chronic atrial fibrillation and experimental congestive heart failure (see Wang *et al*., 2004). The genes for $M_2$ and $M_3$ receptors are expressed in human coronary arteries (Niihashi *et al*., 2000), although the functional importance of these receptors is currently unclear. Studies using knockout mice lacking $M_2$, $M_3$ or $M_5$ receptors (Yamada *et al*., 2001b; Lamping *et al*., 2004) have suggested that $M_3$ receptors predominantly mediate ACh-induced (endothelium-dependent) dilatation of mouse coronary arteries (Lamping *et al*., 2004). Whether this finding extends to human tissue remains to be determined, and data from functional studies using the human coronary artery are awaited.

In summary, available data indicate a prominent role of $M_2$ receptors in cardiac function. Further work is required to elucidate the role of other muscarinic receptor subtypes in the heart and how this may be altered in disease states.

## Determining the ideal antimuscarinic drug for treatment of OAB

The third International Consultation on Incontinence Committee on Drug Therapy reviewed the considerable data supporting the clinical efficacy and safety of antimuscarinic drugs for the treatment of OAB. Following full development programs, darifenacin and solifenacin are the latest agents to enter the market, which includes oxybutynin, propiverine, tolterodine and trospium. There are other historically important but infrequently used drugs with antimuscarinic actions including imipramine (a tricyclic antidepressant with central and peripheral effects), flavoxate (a tertiary amine with calcium antagonistic activity in the bladder), dicyclomine (an antimuscarinic with calcium antagonistic properties) and propantheline (a quaternary amine with anticholinergic activity in the bladder and gastrointestinal tract) (see Andersson *et al*., 2005). However, the latter drugs will not be further discussed in this review.

When identifying the features of the ideal antimuscarinic drug for treatment of OAB, it is important to consider a number of factors. These agents differ with respect to structural characteristics (e.g. trospium is a quaternary ammonium compound, others are tertiary amines), pharmacokinetic profile and mechanism(s) of action (in addition to antimuscarinic action, drugs may also have a calcium channel blocking property). Furthermore, sparing or affecting a particular muscarinic receptor has the potential to be beneficial in terms of tolerability/safety.

Formerly, an ideal antimuscarinic was one that could block the efferent impulses that caused detrusor contraction, without having dose-limiting side effects. Now the ideal drug may also need to have effects on the urothelium and afferent nerves in order to maximize its clinical effectiveness (see Andersson, 2004). The existing drugs have different receptor blocking profiles, but what is not known is whether the more $M_3$ selective blockers have clinical advantages over the less selective drugs. 'Head-to-head' comparative studies between drugs will be needed to resolve the question: 'Which is the best available drug?' However, this question may be difficult to answer until we have more reliable instruments to assess both the symptoms of OAB, such as urgency, and the adverse effects, such as bowel disturbance.

### *Secondary mechanisms of antimuscarinic drug action*

In theory, drugs that have actions in addition to antagonism of muscarinic receptors – such as nonspecified 'direct muscle relaxant effects' (e.g. as attributed to oxybutynin), calcium channel blocking or potassium channel opening properties – could increase effectiveness. Table 2 describes the evidence for the proposed secondary actions for the antimuscarinics in both animal (*in vitro* and *in vivo*) and human studies in OAB (see Andersson, 1984; 1988; Andersson *et al*., 1999; Yono *et al*., 2000; Andersson & Chapple, 2001).

Clearly, such secondary actions can also result in undesirable effects. For example, terodiline – a drug widely perceived by patients and clinicians alike as an effective antimuscarinic – was withdrawn by the regulatory authorities in 1991 owing to its cardiac adverse event profile. This drug possessed calcium channel blocking activity, and induced a specific cardiac arrhythmia known as 'Torsades de Pointes' (see Roden, 2004). By contrast, a clinical study demonstrated that the $M_3$ receptor selective muscarinic antagonist, darifenacin, does not prolong the QT interval and is therefore not expected to cause any harmful effects on cardiac repolarisation (Serra *et al*., 2005).

### *Dosing and pharmacokinetic considerations*

Patient compliance with medication is influenced by a number of factors including dosing schedules (Richter *et al*., 2003). Compliance decreases with increasing number of daily doses, with a pronounced effect noted when more than two doses per day are prescribed (Claxton *et al*., 2001). If the assumption is made that once-daily dosing is optimal, then a single dose needs to provide clinically significant efficacy over a period as close as possible to 24 h. For some patients, treatment given when needed might be preferable, perhaps for 'special occasions' such as socializing. Here, a faster-onset shorter-acting preparation may be useful, although it is important that rapid efficacy is not achieved at the penalty of an unacceptable increase in side effects.

**Table 2** Muscarinic receptor antagonists with secondary mechanisms of action

| *Agent* | *Mechanisms of action* | *Evidence* | *Reference* |
|---|---|---|---|
| Oxybutynin | Muscarinic $M_1/M_3$ receptor antagonist, calcium antagonist and local anesthetic actions | *In vitro* smooth muscle relaxant effect (500 times weaker than antimuscarinic activity)<br>Efficacy in OAB shown in clinical studies<br>Effective on intravesical administration | Reviewed by Andersson & Chapple (2001) |
| Dicyclomine | Nonselective muscarinic receptor antagonist, calcium antagonist action | Efficacy in OAB shown in clinical studies | Reviewed by Andersson *et al.* (1999) |
| Propiverine | Nonselective muscarinic receptor antagonist, calcium antagonist action | Efficacy in OAB shown in clinical studies | Reviewed by Andersson *et al.* (1999) |
| Temiverine | Selective muscarinic $M_3$ receptor antagonist, calcium antagonist action | *In-vitro* inhibition of carbachol- and Ca-induced contractions in human detrusor muscle<br>No published clinical data | Yono *et al.* (2000) |
| Terodiline | Nonselective muscarinic receptor antagonist, calcium antagonistic action | Efficacy in OAB shown in clinical studies<br>Induced ventricular arrhythmias (Torsades de Pointes) | Reviewed by Andersson (1984; 1988) |

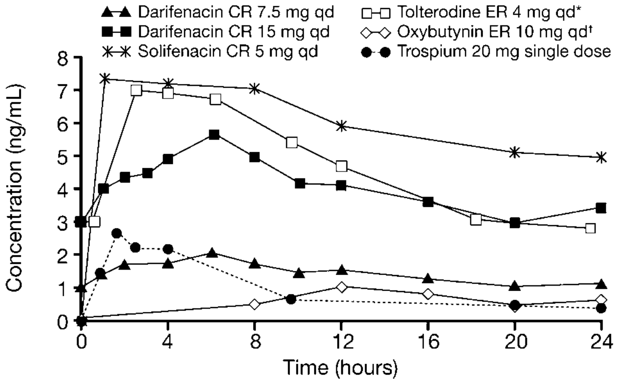

**Figure 2** Mean plasma concentration *versus* time profiles of available antimuscarinic agents (Olsson & Szamosi, 2001; Prescribing Information (Sanctura), 2004; Product Information, Ditropan/Ditropan XL, 2004; Smulders *et al.*, 2004; Product Information, Enablex (US), 2005). *Median serum concentration of active metabolite (5-hydroxymethyl) in healthy volunteers identified as extensive metabolizers. †Mean plasma concentration of R-oxybutynin.

Figure 2 shows the serum concentrations of the commonly available antimuscarinics with curves calculated for oxybutynin and tolterodine ER preparations, trospium 20 mg twice daily, darifenacin 7.5 and 15 mg CR once daily and solifenacin 10 mg once daily (Olsson & Szamosi, 2001; Appell *et al.*, 2003; Prescribing Information, 2004; Smulders *et al.*, 2004; Product Information, Enablex (US), 2005). Of note, owing to the long half-life (40.2 and 49.4 h for the 5 and 10 mg doses, respectively) (Smulders *et al.*, 2004), solifenacin is an outlier in relation to the other drugs. In theory, a longer duration of action following a single dose may be beneficial in smoothing out serum peaks that are believed to increase the prevalence of side effects. However, if the duration of action exceeds 24 h following a single daily dose, then drug accumulation could be an issue. Also, should side effects occur, the patient may have to wait longer before these effects subside. A further downside of a long half-life may be that time to reach steady state is likely to be longer.

Figure 3 shows the difference between the IR and ER versions of oxybutynin and tolterodine in terms of serum concentrations of drug over a 24-h period (Gupta & Sathyan, 1999; Olsson & Szamosi, 2001; Appell *et al.*, 2003). There is some evidence, for each drug, that once-daily dosing of the ER preparation leads to a modest increase in efficacy and a decrease in side effects (Table 3) (Anderson *et al.*, 1999; Gupta & Sathyan, 1999; Appell *et al.*, 2001; Olsson & Szamosi, 2001; Van Kerrebroeck *et al.*, 2001; Appell *et al.*, 2003; Barkin *et al.*, 2004; Product Information (Ditropan/Ditropan XL), 2004; Product Information, Detrol (US), 2005; Product Information, Ditropan (US), 2005). No information is available on the proportion of patients who would prefer to receive treatment when needed rather than as continuous therapy. However, it seems important to preserve the option of an IR version for such individuals.

Thus, there are marked differences in pharmacokinetics between antimuscarinic agents, and some additional parameters are listed for ease of comparison in Table 4 (Douchamps *et al.*, 1988; Prescribing Information (Sanctura), 2004; Prescribing Information, VESIcare (US), 2004; Product Information, Detrol LA (US), 2005; Product Information, Ditropan XL (US), 2005; Product information (Enablex), 2005). Of particular note are the high levels of protein binding reported for most antimuscarinic agents, which would suggest that levels of circulating free drug would be too low to exert pharmacodynamic effect. Despite this, the efficacy of these antimuscarinics is well established. Clearly, therefore, other properties must also be important, and these may include factors such as steady-state levels of receptor occupancy, data on which are not readily available, and the role of active metabolites. The plasma concentration profiles of the active metabolites of tolterodine (4-hydroxymethyltolterodine) and oxybutynin (*N*-desethyloxybutynin) are shown in Figures 2 and 3(a), respectively. Although these clearly exert a pharmacodynamic effect, it is not clear what proportion of total effect may be attributed to the active metabolite *versus* parent molecule.

## *Receptor activity of antimuscarinic agents*

The receptor activity of antimuscarinics can be expressed in different ways, including pharmacologically derived characteristics (Table 5) (Napier & Gupta, 2002) and clinical

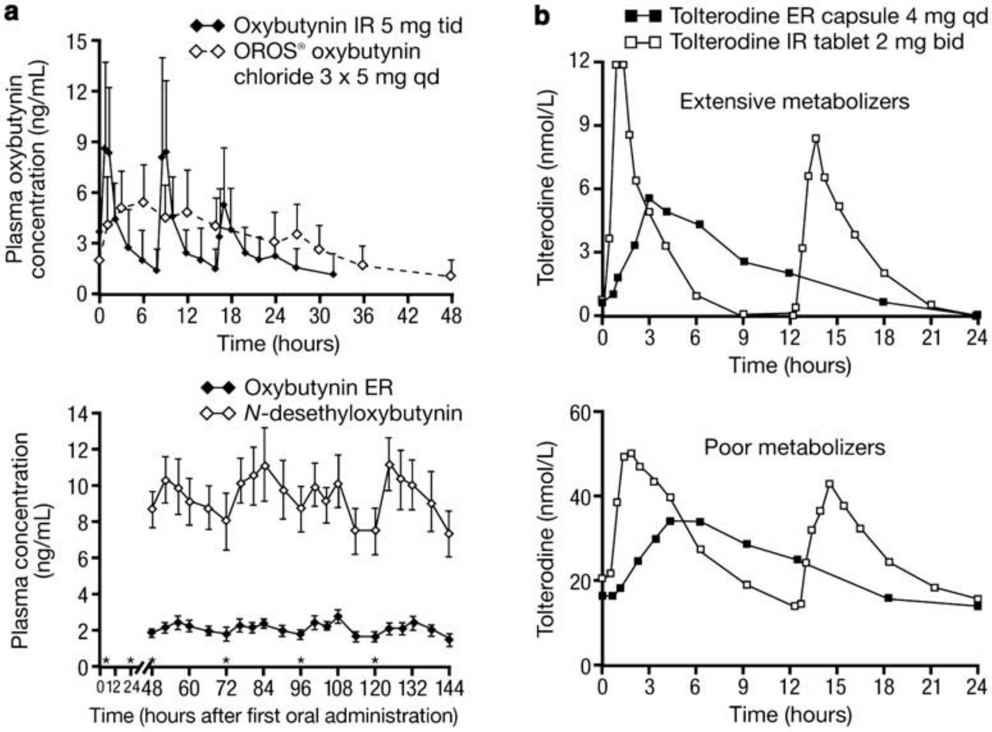

**Figure 3** Mean plasma concentration *versus* time profiles of immediate release and extended release versions of (a) oxybutynin and (b) tolterodine (extensive and poor metabolizers) (Gupta & Sathyan, 1999; Olsson & Szamosi, 2001; Appell *et al*., 2003). *Oral tablet administration. (a) (Upper figure). Reproduced with permission from Gupta S.K. & Sathyan G. Pharmacokinetics of an oral once-a-day controlled-release oxybutynin formulation compared with immediate release oxybutynin. *J. Clin. Pharmacol.* 1999; **39**: 289–296. Copyright 2006, Reprinted by permission of Sage Publication Inc. (Lower figure). Reproduced with permission from Appell RA *et al.* Pharmacokinetics metabolism, and saliva output during transdermal and extended-release oral oxybutynin administration in healthy subjects. *Mayo. Clin. Proc.* 2003; **78**: 696–702. (b) Reproduced with permission from Olsson B *et al.* Multiple dose pharmacokinetics of a new once daily extended release tolterodine formulation *versus* immediate release tolterodine. *Clin. Pharmacokinet.* 2001; **40**: 227–235.

**Table 3** Comparison of safety/efficacy profile of ER and IR formulations of tolterodine and oxybutynin (Anderson *et al*., 1999; Appell *et al*., 2001; Van Kerrebroeck *et al*., 2001; Barkin *et al*., 2004; Product information (Ditropan), 2004; Product Information, Detrol LA (US), 2005; Product Information, Ditropan (US), 2005)

| | *Tolterodine ER 4 mg q.d.* | *Tolterodine IR 2 mg b.i.d.* | *Oxybutynin ER 5–30 mg q.d.* | *Oxybutynin IR 5 mg q.d.–q.i.d.* |
|---|---|---|---|---|
| No. of incontinence episodes, mean change from baseline (%) | –53 | –46 | –64 to –99 | –73 to –88 |
| Incidence of dry mouth (%) | 23 | 35 | 61 | 71.4 |
| Incidence of constipation (%) | 6 | 7 | 13 | 12.6 |

ER, extended-release; IR, immediate release; q.d., once daily; b.i.d., twice daily; q.i.d., four times daily.

uroselectivity. In general, animal models have been used to demonstrate uroselectivity, with studies focusing on how beneficial effects on the bladder predominate over unwanted effects on saliva production (dry mouth) and cardiac effects. Such models demonstrate a wide variation in apparent uroselectivity between drugs (i.e. depending on the model chosen, a selected antimuscarinic may be more uroselective than the others). Nevertheless, such models have been used for the selection of drugs for further development. However, there have been few investigations of uroselectivity performed in humans. One study by Chapple (2001) investigated tolterodine ER (6 mg) and oxybutynin ER (5, 15 and 25 mg) in 16 healthy male volunteers. Tolterodine ER 6 mg produced an increase in bladder capacity comparable with that expected with an oxybutynin ER dose of approximately 20 mg, and a reduction in salivation comparable to that expected with an oxybutynin ER dose of approximately 10 mg.

If it were accepted that the $M_3$ receptor is the only receptor that is important when treating OAB, then it would be expected that a drug that spares other muscarinic receptors would give rise to an optimal tolerability and safety profile. However, the adverse event of constipation associated with the antimuscarinic class of agents might also still be expected.

### *Establishing and comparing risk:benefit profiles*

Adverse event profiling of each drug may shed light on the relationships between beneficial effects and adverse events for each drug. However, to date few studies have been completed that systematically compare the clinical efficacy and safety of the available antimuscarinics. Large clinical trials in patients with OAB have demonstrated clinical efficacy for antimuscarinic agents that differ widely in terms of relative selectivity for the $M_3$ receptor, for example, from the highly $M_3$ receptor selective agent darifenacin (Steers *et al*., 2005) to the relatively nonselective agents tolterodine (see Clemett & Jarvis, 2001) and trospium (Halaska *et al*., 2003). However, comparison of drug profiles using existing clinical study data is difficult due to the lack of standardization of inclusion/exclusion criteria, measurement instruments and the drug dosages used. At present, therefore, it is difficult to draw firm conclusions as to which type of antimuscarinic does, or will, offer the best benefit-to-risk ratio. The recommendations of the International Continence Society's Clinical Trials Standardisation Committee and use of the International Consultation Incontinence modular questionnaire may be helpful. These may enable trials to be conducted that will allow easier comparison between antimuscarinic drugs by making available a standard basic protocol, together with valid instruments to assess outcomes. Nevertheless, a major difficulty will always be how to compare the drugs at similar clinically relevant doses.

**Table 4** Comparison of pharmacokinetic parameters potentially influencing drug availability and activity for selected antimuscarinic agents (Douchamps *et al*., 1988; Prescribing Information (Sanctura), 2004; Prescribing Information, VESIcare (US), 2004; Product information (Enablex), 2005; Product Information, Detrol LA (US), 2005; Product Information, Ditropan XL (US), 2005)

| | *Bioavailability (%)* | *Protein binding (%)* | *Distribution volume at steady state* (l) | *Terminal elimination half-life* (h) |
|---|---|---|---|---|
| Darifenacin | 15–19 | 98 | 163 | 7–20 |
| Tolterodine | ⩾77 | 96 | 113 | 2–10 |
| Oxybutynin | 6 | NA | 193 | 13 |
| Solifenacin | 90 | 98 | 600 | 45–68 |
| Trospium | ⩽10 | 50–85 | 395 | 18 |

NA, not available.

## *Etiology of DO*

Finally, the etiology of DO is another factor that impacts the efficacy of antimuscarinic therapy. Although it is well established that the contractions of the detrusor muscle are in response to the cholinergic stimulation of muscarinic receptors located in the bladder, the exact etiology of DO is largely unknown. The pathophysiology of DO may be neurogenic, myogenic or a combination of both. Neurogenic pathophysiology may possibly involve reduced suprapontine inhibition, damaged axonal paths through the spinal cord, increased afferent input from the lower urinary tract, loss of peripheral inhibition and/or enhanced excitatory neurotransmission in the micturition reflex pathway (see de Groat, 1997). In contrast, myogenic pathophysiology has been postulated to develop following local denervation of bladder smooth muscle leading to increased excitability and easier signal transmissibility between myocytes, thus stimulating the propagation of coordinated contractions (see Turner & Brading, 1997) or micromotions.

Data from guinea-pig studies has also linked DO with inappropriate activation or modulation of autonomous activity *via* suburothelial interstitial cells, resulting in pathological localized contractions and 'sensory urgency' (Gillespie, 2004a). There is also evidence that ACh may be released or leak from postganglionic parasympathetic neurons or from non-neuronal sources during bladder filling, with subsequent micromotions in the detrusor causing an increase in afferent stimulation (see Andersson, 2004).

**Table 5** Comparison of muscarinic receptor affinities and $M_3$ selectivity profiles of antimuscarinic agents (mean binding affinity ratios) (Napier & Gupta, 2002)

(a) *Affinity* ($pK_i$) *of antimuscarinic compounds for the human recombinant receptor subtypes* $M_1$–$M_5$

| | $M_1$ | $M_2$ | $M_3$ | $M_4$ | $M_5$ |
|---|---|---|---|---|---|
| Darifenacin | 8.2 (0.04) | 7.4 (0.10) | 9.1 (0.10) | 7.3 (0.10) | 8.0 (0.10) |
| Tolterodine | 8.8 (0.01) | 8.0 (0.10) | 8.5 (0.10) | 7.7 (0.10) | 7.7 (0.03) |
| Oxybutynin | 8.7 (0.04) | 7.8 (0.10) | 8.9 (0.10) | 8.0 (0.04) | 7.4 (0.03) |
| Propiverine | 6.6 (0.10) | 5.4 (0.10) | 6.4 (0.10) | 6.0 (0.10) | 6.5 (0.10) |
| Trospium | 9.1 (0.10) | 9.2 (0.10) | 9.3 (0.10) | 9.0 (0.10) | 8.6 (0.10) |

(b) *Comparison of the* $M_3$ *selectivity of each compound*

| | $M_3$ *versus* $M_1$ | $M_3$ *versus* $M_2$ | $M_3$ *versus* $M_4$ | $M_3$ *versus* $M_5$ |
|---|---|---|---|---|
| Darifenacin | 9.3*** | 59.2*** | 59.2*** | 12.2*** |
| Tolterodine | 0.6*[a] | 3.6*** | 7.3*** | 6.3*** |
| Oxybutynin | 1.5*[a] | 12.3*** | 6.9*** | 27.0*** |
| Propiverine | 0.6*[a] | 9.6*** | 2.8*** | 0.8 |
| Trospium | 1.5 | 1.3 | 2.0* | 4.6*** |

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$pK_i$ data presented as mean (s.e.m.) ($n = 3$–$6$).

$K_i$ ratios were compared by ANOVA. The ratio of the $K_i$ values in (b) were derived from the antilog of the difference in the mean $pK_i$ values shown in (a).

$^*P<0.05$, $^{***}P<0.001$.

[a] Statistically significant selectivity for $M_1$, although unlikely to be biologically relevant.

## Conclusions

It is well established that muscarinic receptor subtypes are widely distributed throughout the human body, each type having a specific functional and physiological role in each tissue. This distribution of muscarinic receptor subtypes can represent a considerable therapeutic challenge when trying to target receptors specific to an organ system. For example, both in normal bladders and in OAB, detrusor muscle contractions are primarily mediated by stimulation of bladder muscarinic $M_3$ receptors; blockade of $M_3$ receptors can alleviate the symptoms of OAB, although the classic antimuscarinic adverse events of constipation and dry mouth remain. In addition, as evidence is emerging for an indirect role of $M_2$ receptors in detrusor contractility, the potential benefits and risks of $M_2$ receptor antagonism should be further investigated.

Thus, there are a number of important factors that need to be considered when identifying the features of the ideal antimuscarinic drug for treatment of OAB. In the case of OAB, clinical uroselectivity is important (i.e. targeting the bladder) – unwanted effects such as cognitive impairment and blurred vision can only occur if the drug crosses the BBB and blood–retina barrier, respectively; if the drug has a chemical structure which imparts a limited ability to cross these barriers then receptor selectivity in those end organs becomes less of an issue. It remains to be established whether antagonist activity at the $M_3$ receptor subtype, together with blockade of $M_2$ receptors or a secondary nonmuscarinic effect (e.g. direct muscle relaxant effect *via* calcium channel antagonism or potassium channel activation), will increase effectiveness in treating OAB. However, it will be important to establish the risk:benefit ratio for such agents with secondary mechanisms of action. Furthermore, considering the age of patients receiving OAB treatments, it would be preferable to identify those agents that are free of CNS sedation and impairment, and that do not add to the CNS anticholinergic burden.

When considering the pharmacodynamic and pharmacokinetic properties of an antimuscarinic agent, it is important that the pharmacokinetics of the drug (or the formulation of the drug) are such that dosing is once (or no more than twice) daily, as this imparts greatest patient compliance to the dosing schedule.

Antimuscarinic agents relatively selective for the $M_3$ receptor subtype are now available for the treatment of OAB. Current evidence suggests that efficacy observed in pivotal phase III studies with $M_3$ receptor selective agents is comparable to existing less selective agents. Whether $M_3$ relative receptor selectivity can reduce the adverse events and safety concerns theoretically attributed to the untargeted blockade of muscarinic receptors has yet to be determined. Limited data are available that directly compare the efficacy and safety profiles of drugs with differing muscarinic receptor subtype selectivity. In this regard further data are needed.

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## References

ABDEL-RAHMAN, A., SHETTY, A.K. & ABOU-DONIA, M.B. (2002). Disruption of the blood–brain barrier and neuronal cell death in cingulate cortex, dentate gyrus, thalamus, and hypothalamus in a rat model of Gulf-War syndrome. *Neurobiol. Dis.*, **10,** 306–326.

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.

ANAGNOSTARAS, S.G., MURPHY, G.G., HAMILTON, S.E., MITCHELL, S.L., RAHNAMA, N.P., NATHANSON, N.M. & SILVA, A.J. (2003). Selective cognitive dysfunction in acetylcholine $M_1$ muscarinic receptor mutant mice. *Nat. Neurosci.*, **6,** 51–58.

ANDERSON, R.U., MOBLEY, D., BLANK, B., SALTZSTEIN, D., SUSSET, J. & BROWN, J.S. (1999). Once daily controlled *versus* immediate release oxybutynin chloride for urge urinary incontinence. *J. Urol.*, **161,** 1809–1812.

ANDERSSON, K.-E. (1984). Clinical pharmacology of terodiline. *Scand. J. Urol. Nephrol. Suppl.*, **87,** 13–20.

ANDERSSON, K.-E. (1988). Current concepts in the treatment of disorders of micturition. *Drugs,* **35,** 477–494.

ANDERSSON, K.-E. (2004). Antimuscarinics for treatment of overactive bladder. *Lancet Neurol.*, **3,** 46–53.

ANDERSSON, K.E., APPELL, R., AWAD, S., CHAPPLE, C., DRUTZ, H., FINKBEINER, A., HAAB, F. & VELA NAVARRETE, R. (2002). Pharmacological treatment of urinary incontinence. In: *Incontinence, 2nd International Consultation on Incontinence*. ed. Abrams, P., Cardozo, L., Khoury, S. & Wein, A., pp. 479–511. Plymouth, U.K.: Plymbridge Distributors Ltd.

ANDERSSON, K.-E., APPELL, R., CARDOZO, L., CHAPPLE, C., DRUTZ, H., FOURCROY, J., VELA NAVARETTE, R., NISHIZAWA, O. & WEIN, A. (2005). Pharmacological treatment of urinary incontinence. In: *Incontinence, 3rd International Consultation on Incontinence*. ed. Abrams, P., Khoury, S. & Wein, A., Editions 21, pp. 811–854. France: Health Publication Ltd.

ANDERSSON, K.-E., APPELL, R., CARDOZO, L.D., CHAPPLE, C., DRUTZ, H.P., FINKBEINER, A.E., HAAB, F. & VELA NAVARRETE, R. (1999). The pharmacological treatment of urinary incontinence. *BJU Int.*, **84,** 923–947.

ANDERSSON, K.E. & CHAPPLE, C.R. (2001). Oxybutynin and the overactive bladder. *World J. Urol.*, **19,** 319–323.

ANDERSSON, K.-E. & YOSHIDA, M. (2003). Antimuscarinics and the overactive detrusor – which is the main mechanism of action? *Eur. Urol.*, **43,** 1–5.

APPELL, R.A., ABRAMS, P., DRUTZ, H.P., VAN KERREBROECK, P., MILLARD, R. & WEIN, A. (2001). Treatment of overactive bladder: long-term tolerability and efficacy of tolterodine. *World J. Urol.*, **19,** 141–147.

APPELL, R.A., CHANCELLOR, M.B., ZOBRIST, R.H., THOMAS, H. & SANDERS, S.W. (2003). Pharmacokinetics, metabolism, and saliva output during transdermal and extended-release oral oxybutynin administration in healthy subjects. *Mayo Clin. Proc.*, **78,** 696–702.

ARMSTRONG, R.B., LIBER, K.M. & PETERS, K.M. (2005). Comparison of dry mouth in women treated with extended-release formulations of oxybutynin or tolterodine for overactive bladder. *Int. Urol. Nephrol.*, **37,** 247–252.

BALLABH, P., BRAUN, A. & NEDERGAARD, M. (2004). The blood–brain barrier: an overview: structure, regulation, clinical implications. *Neurobiol. Dis.*, **16,** 1–13.

BARKIN, J., CORCOS, J., RADOMSKY, S., JAMMAL, M.-P., MICELI, P.C., REIZ, J.L., HARSANYI, Z. & DARKE, AC. (2004). A randomized, double-blind, parallel-group comparison of controlled- and immediate-release oxybutynin chloride in urge urinary incontinence. *Clin. Ther.*, **26,** 1026–1036.

BAUM, B.J. (1993). Principles of saliva secretion. *Ann. N. Y. Acad. Sci.*, **694,** 17–23.

BEROUKAS, D., GOODFELLOW, R., HISCOCK, J., JONSSON, R., GORDON, T.P. & WATERMAN, S.A. (2002). Up-regulation of M3-muscarinic receptors in labial salivary gland acini in primary Sjögren's syndrome. *Lab. Invest.*, **82,** 203–210.

BIRDER, L.A., BARRICK, S.R., ROPPOLO, J.R., KANAI, A.J., DEGROAT, W.C., KISS, S. & BUFFINGTON, C.A. (2003). Feline interstitial cystitis results in mechanical hypersensitivity and altered ATP release from the bladder urothelium. *Am. J. Physiol. Renal. Physiol.,* **285,** F423–F429.

BODICK, N.C., OFFEN, W.W., LEVEY, A.I., CUTLER, N.R., GAUTHIER, S.G., SATLIN, A., SHANNON, H.E., TOLLEFSON, G.D., RASMUSSEN, K., BYMASTER, F.P., HURLEY, D.J., POTTER, W.Z. & PAUL, S.M. (1997). Effects of xanomeline, a selective muscarinic receptor agonist, on cognitive function and behavioral symptoms in Alzheimer disease. *Arch. Neurol.,* **54,** 465–473.

BOGNAR, I.T., ALTES, U., BEINHAUER, C., KESSLER, I. & FUDER, H. (1992). A muscarinic receptor different from the $M_1$, $M_2$, $M_3$ and $M_4$ subtypes mediates the contraction of the rabbit iris sphincter. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **345,** 611–618.

BOGNAR, I.T., WESNER, M.T. & FUDER, H. (1990). Muscarinic receptor types mediating autoinhibition of acetylcholine release and sphincter contraction in the guinea-pig iris. *Naunyn-Schmiedeberg's Arch Pharmacol.,* **341,** 22–29.

BRAVERMAN, A.S., LUTHIN, G.R. & RUGGIERI, M.R. (1998). $M_2$ muscarinic receptor contributes to contraction of the denervated rat urinary bladder. *Am. J. Physiol.,* **275,** R1654–R1660.

BRAVERMAN, A.S. & RUGGIERI SR, M.R. (2003). Hypertrophy changes the muscarinic receptor subtype mediating bladder contraction from $M_3$ toward $M_2$. *Am. J. Physiol. Regul. Integr. Comp. Physiol.,* **285,** R701–R708.

BYMASTER, F.P., CARTER, P.A., YAMADA, M., GOMEZA, J., WESS, J., HAMILTON, S.E., NATHANSON, N.M., MCKINZIE, D.L. & FELDER, C.C. (2003). Role of specific muscarinic receptor subtypes in cholinergic parasympathomimetic responses, *in vivo* phosphoinositide hydrolysis, and pilocarpine-induced seizure activity. *Eur. J. Neurosci.,* **17,** 1403–1410.

CAULFIELD, M.P. (1993). Muscarinic receptors – characterization, coupling and function. *Pharmacol. Ther.,* **58,** 319–379.

CAULFIELD, M.P. & BIRDSALL, N.J.M. (1998). International union of pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol. Rev.,* **50,** 279–290.

CHAPPLE, C.R. (2000). Muscarinic receptor antagonists in the treatment of overactive bladder. *Urology.,* **55** (Suppl. 5A), 33–46.

CHAPPLE, C.R. (2001). Tolterodine once daily: selectivity for the bladder over effects on salivation compared to Ditropan XL. *J. Urol.,* **165** (5 Suppl), 253 (Abstract 1040).

CHAPPLE, C., STEERS, W., NORTON, P., MILLARD, R., KRALIDIS, G., GLAVIND, K. & ABRAMS, P. (2005). A pooled analysis of three phase III studies to investigate the efficacy, tolerability and safety of darifenacin, a muscarinic $M_3$ selective receptor antagonist, in the treatment of overactive bladder. *BJU Int.,* **95,** 993–1001.

CHAPPLE, C.R. & ABRAMS, P. (2005). Comparison of darifenacin and oxybutynin in patients with overactive bladder: assessment of ambulatory urodynamics and impact on salivary flow. *Eur. Urol.,* **48,** 102–109.

CHESS-WILLIAMS, R. (2002). Muscarinic receptors of the urinary bladder: detrusor, urothelial and prejunctional. *Auton. Autacoid. Pharmacol.,* **22,** 133–145.

CHESS-WILLIAMS, R., CHAPPLE, C.R., YAMANISHI, T., YASUDA, K. & SELLERS, D.J. (2001). The minor population of $M_3$-receptors mediate contraction of human detrusor muscle *in vitro*. *J. Auton. Pharmacol.,* **21,** 243–248.

CHIBA, T., BHARUCHA, A.E., THOMFORDE, G.M., KOST, L.J. & PHILLIPS, S.F. (2002). Model of rapid gastrointestinal transit in dogs: effects of muscarinic antagonists and a nitric oxide synthase inhibitor. *Neurogastroenterol. Motil.,* **14,** 535–541.

CHOPPIN, A. & EGLEN, R.M. (2001). Pharmacological characterization of muscarinic receptors in dog isolated ciliary and urinary bladder smooth muscle. *Br. J. Pharmacol.,* **132,** 835–842.

CHU, F.M., DMCHOWSKI, R.R., LAMA, D.J., ANDERSON, R.U. & SAND, P.K. (2005). Extended-release formulations of oxybutynin and tolterodine exhibit similar central nervous system tolerability profiles: a subanalysis of data from the OPERA trial. *Am. J. Obstet. Gynecol.,* **192,** 1849–1854.

CLAXTON, A.J., CRAMER, J. & PIERCE, C. (2001). A systematic review of the associations between dose regimens and medication compliance. *Clin. Ther.,* **23,** 1296–1310.

CLEMETT, D. & JARVIS, B. (2001). Tolterodine: a review of its use in the treatment of overactive bladder. *Drugs Aging,* **18,** 277–304.

COLECRAFT, H.M., EGAMINO, J.P., SHARMA, V.K. & SHEU, S.-S. (1998). Signaling mechanisms underlying muscarinic receptor-mediated increase in contraction rate in cultured heart cells. *J. Biol. Chem.,* **273,** 32158–32166.

COLLISON, D.J., COLEMAN, R.A., JAMES, R.S., CAREY, J. & DUNCAN, G. (2000). Characterization of muscarinic receptors in human lens cells by pharmacologic and molecular techniques. *Invest. Ophthalmol. Vis. Sci.,* **41,** 2633–2641.

CULP, D.J., LUO, W., RICHARDSON, L.A., WATSON, G.E. & LATCHNEY, L.R. (1996). Both $M_1$ and $M_3$ receptors regulate exocrine secretion by mucous acini. *Am. J. Physiol. Cell Physiol.,* **271,** C1963–C1972.

DE GROAT, W.C. (1997). A neurologic basis for the overactive bladder. *Urology,* **50** (Suppl. 6A), 36–52.

DHEIN, S., VAN KOPPEN, C.J. & BRODDE, O.-E. (2001). Muscarinic receptors in the mammalian heart. *Pharmacol. Res.,* **44,** 161–182.

DIEFENBACH, K., DONATH, F., MAURER, A., BRAVO, S.Q., WERNECKE, K.-D., SCHWANTES, U., HASELMANN, J. & ROOTS, I. (2003). Randomised, double-blind study of the effects of oxybutynin, tolterodine, trospium chloride and placebo on sleep in healthy young volunteers. *Clin. Drug. Invest.,* **23,** 395–404.

DIOKNO, A.C., APPELL, R.A., SAND, P.K., DMOCHOWSKI, R.R., GBUREK, B.M., KLIMBERG, I.W. & KELL, S.H. (2003). Prospective, randomized, double-blind study of the efficacy and tolerability of the extended-release formulations opf oxybutynin and tolterodine for overactive bladder: results of the OPERA trial. *Mayo Clin. Proc.,* **78,** 687–695.

DOBREV, D., KNUSCHKE, D., RICHTER, F., WETTWER, E., CHRIST, T., KNAUT, M. & RAVENS, U. (2003). Functional identification of $M_1$ and $M_3$ muscarinic acetylcholine receptors in human atrial myocytes: influence of chronic atrial fibrillation [abstract]. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **367** (Suppl.1), R94.

DONNELLAN, C.A., FOOK, L. & PLAYFER, J.R. (1997). Oxybutynin and cognitive dysfunction. *BMJ,* **315,** 1363–1364.

DOUCHAMPS, J., DERENNE, F., STOCKIS, A., GANGJI, D., JUVENT, M. & HERCHUELZ, A. (1988). The pharmacokinetics of oxybutynin in man. *Eur. J. Clin. Pharmacol.,* **35,** 515–520.

DUVVURI, S., MAJUMAR, S. & MITRA, A.K. (2003). Drug delivery to the retina: challenges and opportunities. *Expert Opin. Biol. Ther.,* **3,** 45–56.

EGLEN, R.M. (2001). Muscarinic receptors and gastrointestinal tract smooth muscle function. *Life Sci.,* **68,** 2573–2578.

EGLEN, R.M. & HARRIS, G.C. (1993). Selective inactivation of muscarinic $M_2$ and $M_3$ receptors in guinea-pig ileum and atria *in vitro*. *Br. J. Pharmacol.,* **109,** 946–952.

EHLERT, F.J., GRIFFIN, M.T., ABE, D.M., VO, T.H., TAKETO, M.M., MANABE, T. & MATSUI, M. (2005). The $M_2$ muscarinic receptor mediates contraction through indirect mechanisms in mouse urinary bladder. *J. Pharmacol. Exp. Ther.,* **313,** 368–378.

ESPOSITO, P., GHEORGHE, D., KANDERE, K., PANG, X., CONNOLLY, R., JACOBSON, S. & THEOHARIDES, T.C. (2001). Acute stress increases permeability of the blood–brain-barrier through activation of brain mast cells. *Brain Res.,* **888,** 117–127.

FETSCHER, C., FLEICHMAN, M., SCHMIDT, M., KREGE, S. & MICHEL, M.C. (2002). $M_3$ muscarinic receptors mediate contraction of human urinary bladder. *Br. J. Pharmacol.,* **136,** 641–643.

FISHER, A., PITTEL, Z., HARING, R., BAR-NER, N., KLIGER-SPATZ, M., NATAN, N., EGOZI, I., SONEGO, H., MARCOVITCH, I. & BRANDEIS, R. (2003). $M_1$ muscarinic agonists can modulate some of the hallmarks in Alzheimer's disease. *J. Mol. Neurosci.,* **20,** 349–356.

FRY, C.H., IKEDA, Y., HARVEY, R., WU, C. & SUI, G.-P. (2004). Control of bladder function by peripheral nerves: avenues for novel drug targets. *Urology,* **63** (Suppl. 3A), 24–31.

GAUTAM, D., HEARD, T.S., CUI, Y., MILLER, G., BLOODWORTH, L. & WESS, J. (2004). Cholinergic stimulation of salivary secretion studied with $M_1$ and $M_3$ muscarinic receptor single- and double-knockout mice. *Mol. Pharmacol.,* **66,** 260–267.

GERSHON, M.D. (2003). Serotonin and its implication for the management of irritable bowel syndrome. *Rev. Gastroenterol. Disord.,* **3** (Suppl. 2), S25–S34.

GIL, D.W., KRAUSS, H.A., BOGARDUS, A.M. & WOLDEMUSSIE, E. (1997). Muscarinic receptor subtypes in human iris-ciliary body measured by immunoprecipitation. *Invest. Ophthalmol. Vis. Sci.,* **38,** 1434–1442.

GILLBERG, P.G., SUNDQUIST, S. & NILVEBRANT, L. (1998). Comparison of the *in vitro* and *in vivo* profiles of tolterodine with those of subtype-selective muscarinic receptor antagonists. *Eur. J. Pharmacol.,* **349,** 285–292.

GILLESPIE, J.I. (2004a). The autonomous bladder: a view of the origin of bladder overactivity and sensory urge. *BJU Int.,* **93,** 478–483.

GILLESPIE, J.I. (2004b). Modulation of autonomous contractile activity in the isolated whole bladder of the guinea pig. *BJU Int.,* **93,** 393–400.

GILLESPIE, J.I., HARVEY, I.J. & DRAKE, M.J. (2003). Agonist- and nerve-induced phasic activity in the isolated whole bladder of the guinea pig: evidence for two types of bladder activity. *Exp. Physiol.,* **88,** 343–357.

GOMEZ, A., MARTOS, F., BELLIDO, I., MARQUEZ, E., GARCIA, A.J., PAVIA, J. & DE LA CUESTA, F.S. (1992). Muscarinic receptor subtypes in human and rat colon smooth muscle. *Biochem. Pharmacol.,* **43,** 2413–2419.

GOMEZA, J., SHANNON, H., KOSTENIS, E., FELDER, C., ZHANG, L., BRODKIN, J., GRINBERG, A., SHENG, H. & WESS, J. (1999). Pronounced pharmacologic deficits in M2 muscarinic acetylcholine receptor knockout mice. *Proc. Natl. Acad. Sci. U.S.A.,* **96,** 1692–1697.

GOSLING, J.A., DIXON, J.S. & JEN, P.Y. (1999). The distribution of noradrenergic nerves in the human lower urinary tract: a review. *Eur. Urol.,* **36** (Suppl. 1), 23–30.

GUPTA, S.K. & SATHYAN, G. (1999). Pharmacokinetics of an oral once-a-day controlled-release oxybutynin formulation compared with immediate-release oxybutynin. *J. Clin. Pharmacol.,* **39,** 289–296.

HAAB, F., STEWART, L. & DWYER, P. (2004). Darifenacin, an $M_3$ selective receptor antagonist, is an effective and well-tolerated once-daily treatment for overactive bladder. *Eur. Urol.,* **45,** 420–429.

HABGOOD, M.D., BEGLEY, D.J. & ABBOTT, N.J. (2000). Determinants of passive drug entry into the central nervous system. *Cell Mol. Neurobiol.,* **20,** 231–253.

HALASKA, M., RALPH, G., WIEDEMANN, A., PRIMUS, G., BALLERING-BRÜHL, B., HÖFNER, K. & JONAS, U. (2003). Controlled, double-blind, multicentre clinical trial to investigate long-term tolerability and efficacy of trospium chloride in patients with detrusor instability. *World J. Urol.,* **20,** 392–399.

HARDOUIN, S.N., RICHMOND, K.N., ZIMMERMAN, A., HAMILTON, S.E., FEIGL, E.O. & NATHANSON, N.M. (2002). Altered cardiovascular responses in mice lacking the $M_1$ muscarinic acetylcholine receptor. *J. Pharmacol. Exp. Ther.,* **301,** 129–137.

HEGDE, S.S., CHOPPIN, A., BONHAUS, D., BRIAUD, S., LOEB, M., MOY, T.M., LOURY, D. & EGLEN, RM. (1997). Functional role of $M_2$ and $M_3$ muscarinic receptors in the urinary bladder of rats *in vitro* and *in vivo*. *Br. J. Pharmacol.,* **120,** 1409–1418.

HEGDE, S.S. & EGLEN, R.M. (1999). Muscarinic receptor subtypes modulating smooth muscle contractility in the urinary bladder. *Life Sci.,* **64,** 419–428.

HELLGREN, I., MUSTAFA, A., RIAZI, M., SULIMAN, I., SYLVÉN, C. & ADEM, A. (2000). Muscarinic $M_3$ receptor subtype gene expression in the human heart. *Cell Mol. Life Sci.,* **57,** 175–180.

HULME, E.C., BIRDSALL, N.J.M. & BUCKLEY, N.J. (1990). Muscarinic receptor subtypes. *Ann. Rev. Pharmacol. Toxicol.,* **30,** 633–673.

IGAWA, Y., ZHANG, X., NISHIZAWA, O., UMEDA, M., IWATA, A., TAKETO, M.M., MANABE, T., MATSUI, M. & ANDERSSON, K.E. (2004). Cystometric findings and in mice lacking muscarinic $M_2$ or $M_3$ receptors. *J Urol.,* **172,** 2460–2464.

IKEDA, K., KOBAYASHI, S., SUZUKI, M., MIYATA, K., TAKEUCHI, M., YAMADA, T. & HONDA, K. (2002). $M_3$ receptor antagonism by the novel antimuscarinic agent solifenacin in the urinary bladder and salivary gland. *Naunyn-Schmiedeberg's Arch Pharmacol.,* **366,** 97–103.

ISHIZAKA, N., NODA, M., YOKOYAMA, S., KAWASAKI, K., YAMAMOTO, M. & HIGASHIDA, H. (1998). Muscarinic acetylcholine receptor subtypes in the human iris. *Brain Res.,* **787,** 344–347.

ISLAM, M.A., NOJIMA, H. & KIMURA, I. (1998). Muscarinic $M_1$ receptor activation reduces maximum upstroke velocity of action potential in mouse right atria. *Eur. J. Pharmacol.,* **346,** 227–236.

JUMBLATT, J.E. & HACKMILLER, R.C. (1994). $M_2$-type muscarinic receptors mediate prejunctional inhibition of norepinephrine release in the human iris-ciliary body. *Exp. Eye. Res.,* **58,** 175–180.

KANNO, H., HORIKAWA, Y., HODGES, R.R., ZOUKHRI, D., SHATOS, M.A., RIOS, J.D. & DARTT, D.A. (2003). Cholinergic agonists transactivate EGFR and stimulate MAPK to induce goblet cell secretion. *Am. J. Physiol. Cell Physiol.,* **284,** C988–C998.

KATZ, I.R., SANDS, L.P., BILKER, W., DIFILIPPO, S., BOYCE, A. & D'ANGELO, K. (1998). Identification of medications that cause cognitive impairment in older people: the case of oxybutynin chloride. *J. Am. Geriatr. Soc.,* **46,** 8–13.

KAY, G. & WESNES, K. (2005). Pharmacodynamic effects of darifenacin, a muscarinic $M_3$ selective antagonist for the treatment of overactive bladder, in healthy volunteers. *BJU Int.,* **96,** 1055–1062.

KERR, P.M., HILLIER, K., WALLIS, R.M. & GARLAND, C.J. (1995). Characterization of muscarinic receptors mediating contractions of circular and longitudinal muscle of human isolated colon. *Br. J. Pharmacol.,* **115,** 1518–1524.

KRICHEVSKY, V.P., PAGALA, M.K., VAYDOVSKY, I., DAMER, V. & WISE, G.J. (1999). Function of $M_3$ muscarinic receptors in the rat urinary bladder following partial outlet obstruction. *J. Urol.,* **161,** 1644–1650.

LAMPING, K.G., WESS, J., CUI, Y., NUNO, D.W. & FARACI, F.M. (2004). Muscarinic (M) receptors in coronary circulation: gene-targeted mice define the role of $M_2$ and $M_3$ receptors in response to acetylcholine. *Arterioscler. Thromb. Vasc. Biol.,* **24,** 1253–1258.

LI, M., JOHNSON, C.P., ADAMS, M.B. & SARNA, S.K. (2002). Cholinergic and nitrergic regulation of *in vivo* giant migrating contractions in rat colon. *Am. J. Physiol. Gastrointest. Liver. Physiol.,* **283,** G544–G552.

LIEBSCH, R., KORNHUBER, M.E., DIETL, D., GRÄFIN VON EINSIEDEL, H. & CONRAD, B. (1996). Blood–CSF barrier integrity in multiple sclerosis. *Acta Neurol. Scand.,* **94,** 404–410.

LIPTON, R.B., KOLODNER, K. & WESNES, K. (2005). Assessment of cognitive function of the elderly population: effects of darifenacin. *J. Urol.,* **173,** 493–498.

LUO, W., LATCHNEY, L.R. & CULP, D.J. (2001). G protein coupling to $M_1$ and $M_3$ muscarinic receptors in sublingual glands. *Am. J. Physiol. Cell Physiol.,* **280,** C884–C896.

MATSUI, M., MOTOMURA, D., FUJIKAWA, T., JIANG, J., TAKAHASHI, S., MANABE, T. & TAKETO, M.M. (2002). Mice lacking $M_2$ and $M_3$ muscarinic acetylcholine receptors are devoid of cholinergic smooth muscle contractions but still viable. *J. Neurosci.,* **22,** 10627–10632.

MATSUI, M., MOTOMURA, D., KARASAWA, H., FUJIKAWA, T., JIANG, J., KOMIYA, Y., TAKAHASHI, S. & TAKETO, M.M. (2000). Multiple functional defects in peripheral autonomic organs in mice lacking muscarinic acetylcholine receptor gene for the $M_3$ subtype. *Proc. Natl. Acad. Sci. U.S.A.,* **97,** 9579–9584.

MESSER JR, W.S., BOHNETT, M. & STIBBE, J. (1990). Evidence for a preferential involvement of $M_1$ muscarinic receptors in representational memory. *Neurosci. Lett.,* **116,** 184–189.

NAPIER, C. & GUPTA, P. (2002). Darifenacin is selective for the human recombinant $M_3$ receptor subtype [abstract]. *Neurourol. Urodyn.,* **21,** A445.

NIIHASHI, M., ESUMI, M., KUSUMI, Y., SATO, Y. & SAKURAI, I. (2000). Expression of muscarinic receptor genes in the human coronary artery. *Angiology.,* **51,** 295–300.

NISHIMARU, K., TANAKA, Y., TANAKA, H. & SHIGENOBU, K. (2000). Positive and negative inotropic effects of muscarinic receptor stimulation in mouse left atria. *Life Sci.,* **66,** 607–615.

OLSSON, B. & SZAMOSI, J. (2001). Multiple dose pharmacokinetics of a new once daily extended release tolterodine formulation *versus* immediate release tolterodine. *Clin. Pharmacokinet.,* **40,** 227–235.

PAKULSKI, C., DROBNIK, L. & MILLO, B. (2000). Age and sex as factors modifying the function of the blood–cerebrospinal fluid barrier. *Med. Sci. Monit.,* **6,** 314–318.

PERRY, E.K., KILFORD, L., LEES, A.J., BURN, D.J. & PERRY, R.H. (2003). Increased Alzheimer pathology in Parkinson's disease related to antimuscarinic drugs. *Ann Neurol.,* **54,** 235–238.

PIETZKO, A., DIMPFEL, W., SCHWANTES, U. & TOPFMEIER, P. (1994). Influences of trospium chloride and oxybutynin on quantitative EEG in healthy volunteers. *Eur. J. Clin. Pharmacol.,* **47,** 337–343.

PÖNICKE, K., HEINROTH-HOFFMANN, I. & BRODDE, O.-E. (2003). Demonstration of functional $M_3$-muscarinic receptors in ventricular cardiomyocytes of adult rats. *Br. J. Pharmacol.,* **138,** 156–160.

PONTARI, M.A., BRAVERMAN, A.S. & RUGGIERI SR, M.R. (2004). The $M_2$ muscarinic receptor mediates *in vitro* bladder contractions from patients with neurogenic bladder dysfunction. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **286,** R874–R880.

POYER, J.F., GABELT, B.T. & KAUFMAN, P.L. (1994). The effect of muscarinic agonists and selective receptor subtype antagonists on the contractile response of the isolated Rhesus monkey ciliary muscle. *Exp. Eye Res.*, **59,** 729–736.

PRESCRIBING INFORMATION (2004). Sanctura trospium chloride 20 mg tablets, http://www.sanctura.com/Sanctura_Prescribing_Information.pdf.

PRESCRIBING INFORMATION, VESICARE (US) (2004) http://www.vesicare.com/pdf/vesicareprescribing_info.pdf.

PRODUCT INFORMATION, DETROL (US) (2005) http://www.pfizer.com/download/uspi_detrol.pdf.

PRODUCT INFORMATION, DETROL LA (US) (2005) http://pfizer.com/pfizer/download/uspi_detrol_la.pdf.

PRODUCT INFORMATION, ENABLEX (US) (2005) http://www.pharma.us.novartis.com/product/pi/pdf/enablex.pdf.

PRODUCT INFORMATION, DITROPAN (US) (2005) http://www.ditropanxl.com/professional/full_presc.htm.

PRODUCT INFORMATION. DITROPAN/DITROPAN XL (2004) http://www.orthomcneil.com/products/pi/pdfs/Lg%20Ditropan%20PI.pdf, http://www.orthomcneil.com/products/pi/pdfs/ditropanxl.pdf.

RICHTER, A., ANTON, S.E., KOCH, P. & DENNETT, S.L. (2003). The impact of reducing dose frequency on health outcomes. *Clin. Ther.*, **25,** 2307–2335.

RODEN, D.M. (2004). Drug-induced prolongation of the QT interval. *N. Engl. J. Med.*, **350,** 1013–1022.

SEEGER, T., FEDOROVA, I., ZHENG, F., MIYAKAWA, T., KOUSTOVA, E., GOMEZA, J., BASILE, A.S., ALZHEIMER, C. & WESS, J. (2004). $M_2$ muscarinic acetylcholine receptor knock-out mice show deficits in behavioral flexibility, working memory, and hippocampal plasticity. *J. Neurosci.*, **24,** 10117–10127.

SERRA, D.B., AFFRIME, M.B., BEDIGIAN, M.P., GREIG, G., MILOSAVLJEV, S., SKERJANEL, A. & WANG, Y. (2005). QT and QTc interval with standard and supra-therapeutic doses of darifenacin, a muscarinic $M_3$ selective receptor antagonist for the treatment of overactive bladder. *J. Clin. Pharm.*, **45,** 1038–1047.

SHADE, D.L., CLARK, A.F. & PANG, I.-H. (1996). Effects of muscarinic agents on cultured human trabecular meshwork cells. *Exp. Eye Res.*, **62,** 201–210.

SMULDERS, R.A., KRAUWINKEL, W.J., SWART, P.J. & HUANG, M. (2004). Pharmacokinetics and safety of solifenacin succinate in healthy young men. *J. Clin. Pharmacol.*, **44,** 1023–1033.

SO, I., YANG, D.K., KIM, H.J., MIN, K.W., KANGE, T.M., KIM, S.J., KIM, K.W., PARTK, K.H., JEON, J.H., CHOI, K.H. & KIM, I.G. (2003). Five subtypes of muscarinic receptors are expressed in gastric smooth muscles of guinea pig. *Exp. Mol. Med.*, **35,** 46–52.

SOMOGYI, G.T. & DE GROAT, W.C. (1999). Function, signal transduction mechanisms and plasticity of presynaptic muscarinic receptors in the urinary bladder. *Life Sci.*, **64,** 411–418.

SOMOGYI, G.T., TANOWITZ, M., ZERNOVA, G. & DE GROAT, W.C. (1996). $M_1$ muscarinic receptor -induced facilitation of ACh and noradrenaline release in the rat bladder is mediated by protein kinase C. *J. Physiol.*, **496,** 245–254.

SOMOGYI, G.T., ZERNOVA, G.V., YOSHIYAMA, M., ROCHA, J.N., SMITH, C.P. & DE GROAT, W.C. (2003). Change in muscarinic modulation of transmitter release in the rat urinary bladder after spinal cord injury. *Neurochem. Int.*, **43,** 73–77.

STARR, J.M., WARDLAW, J., FERGUSON, K., MACLULLICH, A., DEARY, I.J. & MARSHALL, I. (2003). Increased blood–brain barrier permeability in type II diabetes demonstrated by gadolinium magnetic resonance imaging. *J. Neurol. Neurosurg. Psychiatry.*, **74,** 70–76.

STEERS, W., CORCOS, J., FOOTE, J. & KRALIDIS, G. (2005). An investigation of dose titration with darifenacin, an $M_3$ selective receptor antagonist. *BJU Int.*, **95,** 580–586; Erratum in: *BJU Int.*, **95,** 1385–1386.

STENGEL, P.W., GOMEZA, J., WESS, J. & COHEN, M.L. (2000). $M_2$ and $M_4$ receptor knockout mice: muscarinic receptor function in cardiac and smooth muscle *in vitro*. *J. Pharmacol. Exp. Ther.*, **292,** 877–885.

STENGEL, P.W., YAMADA, M., WESS, J. & COHEN, M.L. (2002). $M_3$-receptor knockout mice: muscarinic receptor function in atria, stomach fundus, urinary bladder, and trachea. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **282,** R1443–R1449.

STEVENS, L., CHAPPLE, C.R., TOPHILL, P. & CHESS-WILLIAMS, R. (2004a). A comparison of muscarinic receptor-mediated function in the normal and the neurogenic overactive bladder. *J Urol.*, **171,** 143 (Abstract 535).

STEVENS, L., CHESS-WILLIAMS, R. & CHAPPLE, C.R. (2004b). Muscarinic receptor function in the idiopathic overactive bladder. *J Urol.*, **171,** 140–141 (Abstract 527).

THIEME, H., HILDEBRANDT, J., CHORITZ, L., STRAUSS, O. & WIEDERHOLT, M. (2001). Muscarinic receptors of the M2 subtype in human and bovine trabecular meshwork. *Graefe's Arch. Clin. Exp. Ophthalmol.*, **239,** 310–315.

THOMAS, S., ROMANZI, L. & LHERITIER, K. (2005). Constipation and associated intervention in patients with overactive bladder treated with darifenacin or tolterodine. *Int. Urogynecol. J.*, **16** (Suppl. 2), S101 (Abstract 306).

TOBIN, G. (2002). Presynaptic muscarinic receptor mechanisms and submandibular responses to stimulation of the parasympathetic innervation in bursts in rats. *Auton. Neurosci.*, **99,** 111–118.

TOBIN, G., GIGLIO, D. & GÖTRICK, B. (2002). Studies of muscarinic receptor subtypes in salivary gland function in anaesthetized rats. *Auton. Neurosci.*, **100,** 1–9.

TRENDELENBURG, A.-U., GOMEZA, J., KLEBROFF, W., ZHOU, H. & WESS, J. (2003). Heterogeneity of presynaptic muscarinic receptors mediating inhibition of sympathetic transmitter release: a study with $M_2$- and $M_4$-receptor-deficient mice. *Br. J. Pharmacol.*, **138,** 469–480.

TUNE, L.E. (2001). Anticholinergic effects of medication in elderly patients. *J. Clin. Psychiatry*, **62** (Suppl. 21), 11–14.

TURNER, W.H. & BRADING, A.F. (1997). Smooth muscle of the bladder in the normal and the diseased state: pathophysiology, diagnosis and treatment. *Pharmacol. Ther.*, **75,** 77–110.

TZAVARA, E.T., BYMASTER, F.P., FELDER, C.C., WADE, M., GOMEZA, J., WESS, J., MCKINZIE, D.L. & NOMIKOS, G.G. (2003). Dysregulated hippocampal acetylcholine neurotransmission and impaired cognition in $M_2$, $M_4$ and $M_2/M_4$ muscarinic receptor knockout mice. *Mol. Psychiatry*, **8,** 673–679.

VAN KERREBROECK, P., KREDER, K., JONAS, U., ZINNER, N. & WEIN, A. (2001). Tolterodine once-daily: superior efficacy and tolerability in the treatment of the overactive bladder. *Urology*, **57,** 414–421.

VOLPICELLI, L.A. & LEVEY, A.I. (2004). Muscarinic acetylcholine receptor subtypes in cerebral cortex and hippocampus. *Prog. Brain Res.*, **145,** 59–66.

WANG, H., HAN, H., ZHANG, L., SHI, H., SCHRAM, G., NATTEL, S. & WANG, Z. (2001). Expression of multiple subtypes of muscarinic receptors and cellular distribution in the human heart. *Mol. Pharmacol.*, **59,** 1029–1036.

WANG, P., LUTHIN, G.R. & RUGGIERI, M.R. (1995). Muscarinic acetylcholine receptor subtypes mediating urinary bladder contractility and coupling to GTP binding proteins. *J. Pharmacol. Exp. Ther.*, **273,** 959–966.

WANG, Z., SHI, H. & WANG, H. (2004). Functional $M_3$ muscarinic acetylcholine receptors in mammalian hearts. *Br. J. Pharmacol.*, **142,** 395–408.

WATSON, E.L., ABEL, P.W., DIJULIO, D., ZENG, W., MAKOID, M., JACOBSON, K.L., POTTER, L.T. & DOWD, F.J. (1996). Identification of muscarinic receptor subtypes in mouse parotid gland. *Am. J. Physiol. Cell Physiol.*, **271,** C905–C913.

WIEDERHOLT, M., SCHÄFER, R., WAGNER, U. & LEPPLE-WIENHUES, A. (1996). Contractile response of the isolated trabecular meshwork and ciliary muscle to cholinergic and adrenergic agents. *German J. Ophthalmol.*, **5,** 146–153.

WILLMY-MATTHES, P., LEINEWEBER, K., WANGEMANN, T., SILBER, R.E. & BRODDE, O.-E. (2003). Existence of functional $M_3$-muscarinic receptors in the human heart. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **368,** 316–319.

WOLDEMUSSIE, E., FELDMANN, B.J. & CHEN, J. (1993). Characterization of muscarinic receptors in cultured human iris sphincter and ciliary smooth muscle cells. *Exp. Eye Res.*, **56,** 385–392.

WOMACK, K.B. & HEILMAN, K.M. (2003). Tolterodine and memory: dry but forgetful. *Arch. Neurol.*, **60,** 771–773.

YAMADA, M., MIYAKAWA, T., DUTTAROY, A., YAMANAKA, A., MORIGUCHI, T., MAKITA, R., MCKINZIE, D.L., FELDER, C.C., DENG, C.X., FARACI, F.M. & WESS, J. (2001a). Mice lacking the $M_3$ muscarinic acetylcholine receptor are hypophagic and lean. *Nature,* **410,** 207–212.

YAMADA, M., LAMPING, K.G., DUTTAROY, A., ZHANG, W., CUI, Y., BYMASTER, F.P., OGAWA, M., CHOU, C.J., XIA, B., CRAWLEY, J.N., FELDER, C.C., DENG, C.X. & WESS, J. (2001b). Cholinergic dilation of cerebral blood vessels is abolished in $M_5$ muscarinic acetylcholine receptor knockout mice. *Proc. Natl. Acad. Sci. U.S.A.,* **98,** 14096–14101.

YONO, M., YOSHIDA, M., TAKAHASHI, W., INADOME, A. & UEDA, S. (2000). Comparison of the effects of novel antimuscarinic drugs on human detrusor smooth muscle. *BJU Int.,* **86,** 719–725.

YOSHIDA, M., MIYAMAE, K., IWASHITA, H., OTANI, M. & INADOME, A. (2004). Management of detrusor dysfunction in the elderly: changes in acetylcholine and adenosine triphosphate release during aging. *Urology,* **63** (3 Suppl. 1), 17–23.

ZHOU, H., MEYER, A., STARKE, K., GOMEZA, J., WESS, J. & TRENDELENBERG, A.U. (2002). Heterogeneity of release-inhibiting muscarinic autoreceptors in heart atria and urinary bladder: a study with M(2)- and M(4)-receptor-deficient mice. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **365,** 112–122.

ZINNER, N., TUTTLE, J. & MARKS, L. (2005). Efficacy and tolerability of darifenacin, a muscarinic $M_3$ selective receptor antagonist ($M_3$ SRA), compared with oxybutynin in the treatment of patients with overactive bladder. *World J. Urol.,* **23,** 248–252.

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