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TOPIC PAPER

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Which muscarinic receptor is important in the bladder?

Abstract Antimuscarinic agents are the most widely used therapy for urge incontinence, but have side effects such as constipation, tachycardia and dry mouth, resulting from a lack of selectivity for the bladder. M_2 receptors are the predominant cholinoceptors present in urinary bladder, but mainly the minor population of M_3 receptors mediate its contraction. M_2 receptors modulate detrusor contraction by several mechanisms, and may contribute more to contraction of the bladder in pathological states such as bladder denervation or spinal cord injury. Prejunctional inhibitory M_2 or M_4 receptors and prejunctional facilitatory muscarinic M_1 receptors in the bladder have all been reported. In clinical studies, tolterodine, a non-selective muscarinic antagonist, has been reported to be as effective as oxybutynin but inducing less dry mouth. Thus, although it is not certain which antimuscarinic drugs have the better efficacy and tolerability, the non-selective antimuscarinic drugs seem to be better than M_3-selective antagonists in their clinical efficacies. However, controlled release, or intravesical, intravaginal, or rectal administrations of oxybutynin have been reported to cause fewer side effects. Darifenacin, a new M_3 selective antagonist, has been reported to have selectivity for the bladder over the salivary gland in vivo. To verify which antimuscarinic drugs selective for the muscarinic subtypes have the best efficacy and tolerability, comparative clinical trials between M_3 selective antagonists and non-selective compounds, such as olterodine, are required in the future.

Key words muscarinic receptor · urinary bladder · urinary incontinence · therapy

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The lower urinary tract has two functions, i.e. storing and emptying urine. Failure to store urine may lead to various forms of incontinence (mainly urge and stress urinary incontinence). A 33–61% prevalence of an overactive bladder in the elderly over the age of 65 years old has been reported [5]. Bladder contraction is predominantly under the control of the parasympathetic nervous system where input is through muscarinic receptors [5, 37].

Antimuscarinic preparations are the most widely used treatment agents for urge incontinence, but these have side effects including accommodation paralysis, constipation, tachycardia, and dryness of mouth [5]. These side effects results from a lack of selectivity for the bladder resulting in actions on other organs such as the iris, intestine, and salivary gland.

Which muscarinic receptor subtypes are present in the urinary bladder?

The urinary bladder receives cholinergic innervation via the pelvic nerves and adrenergic innervation via the hypogastric nerves. The density of muscarinic receptors is greater in the bladder body than in the base, and cholinergic stimulation produces a contraction of the bladder body of significantly greater magnitude than that of the bladder base [38].

Five different muscarinic subtypes (currently upper case nomenclature M_1–M_5 has been recommended rather than m_1–m_5) have been cloned, and M_1–M_4

subtypes correlate well with the M_1–M_4 gene products pharmacologically [15]. M_1 receptors prevail in neuronal tissues (cerebral cortex, hippocampus, sympathetic ganglia) and are also present in glands. M_2 receptors are present in the heart, hindbrain and smooth muscle. M_3 receptors prevail in exocrine glands and are also found in smooth muscle and the brain. M_4 receptors are found in the basal forebrain and striatum. It has been reported that M_5 receptors are expressed in the substantia nigra [15]. A pharmacological correlate of the M_5 gene has been defined, but because of the similarity in pharmacological profiles of M_5 and M_3 subtypes and the current lack of a high affinity M_5 selective antagonist, the identification of a functional correlate has been complicated [15, 21, 74].

The urinary bladder, like the majority of other smooth muscles from many species exhibit heterogeneous populations of muscarinic receptors [29, 40]. In studies employing northern blot hybridization analysis, the presence of mRNA encoding the M_2 and M_3 subtypes, but not the M_1, M_2, M_4 receptors, has been identified in the bladder of the rat and pig [42]. In the reverse transcriptase-polymerase chain reaction experiments the presence of only M_2 and M_3 subtypes, with a ratio of 1.06:1 has been detected in human urinary bladder [76], but Braverman et al. [9] identified the presence of M_1, M_2, M_3, and M_4 transcripts in rat bladder. At protein level using receptor binding, M_1, M_2, and M_3-receptors have been detected in human detrusor muscle [35], but other studies have detected only M_2 receptors [25] or a mixed M_2 and M_3 receptor population [47, 77]. Similarly in immunoprecipitation studies, only the M_2 and M_3 subtypes have been precipitated in rat, rabbit, guinea pig, and human [73].

Thus, a predominance of the M_2 muscarinic receptor subtype, with a minor population of M_3-receptors has been reported for urinary bladder smooth muscle for several species. Immunoprecipitation data, subtype-selective antisera, and radioligand binding studies all indicate that the proportion of muscarinic M_2 and M_3 receptors is approximately 9:1 in the rat bladder [47, 70, 73], and approximately 3:1 in bladders of humans, guinea pigs, rabbits, and pigs [77, 73].

The functional role of muscarinic receptor subtypes for urinary bladder in vitro

M3-muscarinic receptors

Elucidation of the muscarinic receptor subtypes responsible for mediating detrusor responses to cholinoceptor agonists has been hampered by the lack of subtype-selective agonists and antagonists [40]. However, pharmacological characterisation of muscarinic receptors mediating contraction of detrusor muscle in rat [40], rabbit [16, 48], guinea-pig [52], pig [58], and human [20, 78] bladder suggests the singular involvement of M_3 receptors. The best correlation between the antagonist affinities at the muscarinic receptor in rabbit [16], pig [58] and human [19, 78] bladders and the affinities at human recombinant receptors has been obtained at M_3 receptors. A significant correlation has also been found with the M_5 receptors which reflects the lack of selective muscarinic antagonists which can discriminate M_3 and M_5 subtypes [16]. However, the correlation is better at M_3 than M_5 subtypes when using darifenacin and oxybutynin [58, 78], agents which display some selectivity for M_3 receptors over the M_5 subtype [21, 74]. Furthermore, the M_5 gene has not been identified in the bladder [9, 76, 73]. The predominant role of the M_3 subtype in mediating contraction of the bladder has been confirmed by the experiments using mutant mice lacking the receptor gene for the M_3 subtype [44]. Although the hormonal state and gender may influence the sensitivity of the bladder to muscarinic stimulation, there are no differences in the affinity (pA_2) values of muscarinic antagonists, indicating that M_3 receptors mediate the contraction of the rat urinary bladder [39].

These observations suggest that it is a minor population of M_3-receptors which mediates contraction of the detrusor muscle and that M_2 receptors are not directly involved in contraction. Muscarinic M_3-receptor stimulation has been shown to stimulate phosphoinositide hydrolysis causing release of intracellular calcium in guinea pig [52] and human [4, 27] bladder, and this is most likely the signaling mechanism responsible for the direct contractile responses to muscarinic agonists in this tissue [29] (Fig. 1).

Dry mouth being the most common,the adverse effectsof antimuscarinic drugs in the treatment of overactive bladdermay lead to withdrawal of medication [1]. Thus, drugs which have more selectivity for the bladder is desirable. Because M_3 receptor subtypes have been reported to mediate salivary gland secretion, drugs selective for M_3 receptors have been considered to cause dry mouth. Indeed, oxybutynin, a selective M_3 receptor antagonist, has caused more dry mouth than tolterodine, a non-selective antimuscarinic agent [1, 6]. In radioligand binding studies, oxybutynin has been reported to have a higher affinity for muscarinic receptors in the parotid gland than in the bladder, and darifenacin (a M_3 selective antagonist) a two fold higher affinity in parotid gland than in the guinea pig bladder [24] (Table 1). However, Wallis and Napier [71] have reported that darifenacin exhibits functional tissue selectivity for intestinal smooth muscle over the salivary gland. They have also suggested a role for M_5 receptors in the control of salivary secretion [71], although this has been disputed [21].

M_2-muscarinic receptors

M_2 receptors couple to the pertussis toxin-sensitive guanine nucleotide regulatory protein Gi and inhibit adenylyl cyclase activation [14, 22, 26, 64] (Fig. 1). Although

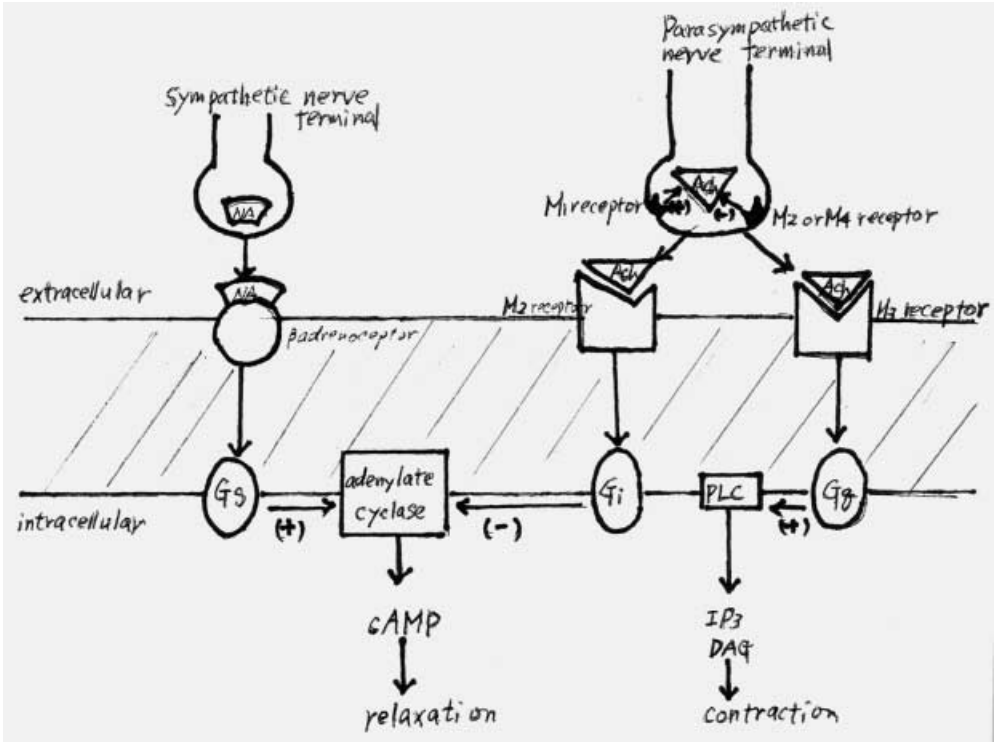

Fig. 1 The role of prejunctional and postjunctional muscarinic receptor subtypes in urinary bladder. *NA* noradrenaline, *ACh* acetylcholine, *PLC* phospholipase C, *cAMP* adenosine 3′5′-cyclic monophosphate, IP_3 inositol (1,4,5)-triphosphate, *DAG* diacylglycerol

no direct contractile response of detrusor smooth muscle to M_2 receptor activation can be demonstrated, an indirect influence on contraction via inhibition of cAMP-mediated smooth muscle relaxation by β-adrenoceptors, forskolin, 5-hydroxytryptamine, or vasoactive intestinal peptide has been reported [14, 23, 26, 53].

M_2-receptors have also been shown to activate smooth muscle by decreasing the probability of opening K^+-channels [8, 49]. Other postulated mechanisms of M_2-mediated contraction include opening of non-specific cation channels resulting in depolarisation and calcium influx, and stimulation of Rho proteins causing Ca^{2+} sensitization [15, 28, 33, 66].

Recently, an M_2-mediated contraction from muscarinic stimulation has been demonstrated following selective M_3-receptor inactivation and elevation of cAMP levels in guinea-pig ileum [53, 64], and in the rat [29] and pig [77] bladder, an effect which manifests as a re-contraction.

Thus, M_2-receptors may mediate the dominant parasympathetic control over smooth muscle tone under conditions of high sympathetic activity or where M_3-receptors are dysfunctional [22]. β-Adrenoceptors predominate over α-adrenoceptors in the urinary bladder body, where their tonic stimulation is thought to facilitate the storage phase of micturition by relaxing the detrusor smooth muscle [38]. Cholinergic activity is inhibited during this filling phase. In contrast, during the voiding phase, sympathetic nerve activity is inhibited and acetylcholine is released. Activation of M_2 receptors during the voiding phase may oppose inhibitory sympathetic activation via β-adrenoceptors, resulting in more efficient bladder emptying or initiation of voiding [40, 51, 77]. Furthermore, M_2-receptors have been reported to directly contribute to contraction of the rat denervated urinary bladder following bilateral major pelvic ganglion removal or spinal cord compression at T9 level [10, 11]. However, Krichevsky et al. [36] have reported that the affinity (pA_2) values for the M_3-selective antagonist 4-DAMP are not significantly different in normal and obstructed rat urinary bladder, suggesting that M_3-receptors still mediate contraction of the bladder with bladder outlet obstruction.

Recently, the role of M_2 and M_3 receptors in mediating contraction of the urinary bladder has been confirmed in experiments using muscarinic receptor subtype knockout mice. Stengel et al. [62] have reported that carbachol-induced bladder contractions in the M_4 receptor knockout mice are similar to wild-type littermates, suggesting that M_4 receptors do not participate in

Table 1 Affinity estimates (pKis) of antagonists in radioligand binding assays at recombinant human muscarinic receptor subtypes expressed in CHO cells. Values are mean Kis estimated in a Tris-Krebs

Antagonist	M_1	M_2	M_3	M_4	M_5
Atropine[a]	9.1	8.9	9.5	9.2	9.1
4-DAMP[a]	9.2	8.1	9.3	8.4	8.9
Darifenacin[a]	7.8	7.0	8.9	7.7	8.1
Methoctramine[a]	6.6	7.6	6.1	6.9	6.4
Oxybutynin[b]	8.5	7.8	8.7	8.2	7.6
Pirenzepine[a]	8.0	6.3	6.8	7.0	6.9
Tolterodine[b]	8.5	8.4	8.5	8.1	8.6

[a] Data from Hegde et al [28]
[b] Data from Eglen and Nahorski [21]

bladder contraction. In contrast, in urinary bladders from M_2 receptor knockout mice, the potency of carbachol was significantly reduced by a factor of ~2, and the affinity of AF-DX116 (an M_2 selective antagonist) in inhibiting carbachol-induced bladder was significantly reduced compared with wild-type littermates. The authors concluded that M_3 receptors are the predominant muscarinic subtype that mediate contractions of smooth muscle including the urinary bladder, and that M_2 but not M_4 receptors also play a role in carbachol-induced contractions of the bladder.

Matsui et al. [44] have reported that mutant mice lacking the receptor gene for the M_3 subtype show impaired salivary secretion to cholinergic stimuli, urinary retention in male mutants, and greatly reduced detrusor contractions from carbachol stimulation (5% of those obtained in the wild-type muscles), suggesting that M_3 receptors play key roles in salivary secretion and bladder contraction. They also reported that the potency of a number of antagonists that block the residual carbachol-induced contractions in these mutant mice is consistent with M_2 receptors in both sexes, suggesting that the M_2 receptors directly mediate the residual contraction of the detrusor of the M_3-knockout mice.

The functional role of muscarinic receptor subtypes in vivo

Although many investigators report that M_3 receptors are the predominant receptor subtype mediating contraction of the bladder in vitro [19, 58, 78], the role of the other muscarinic receptor subtypes in vivo remains to be established. Hegde et al. [29] have reported that the in vivo potency of muscarinic antagonists in rat bladder correlates best with the affinity of such agents for M_2 receptors, and that pretreatment with propranolol (a β-receptor antagonist) decreases the potency of methoctramine (an M_2 receptor antagonist) but not that of darifenacin (an M_3 receptor antagonist) implying an obligatory role for β-adrenoceptors in M_2-receptor mediated effects.

Nilvebrant et al. [51] and Gillberg et al. [24] have shown that tolterodine (a non-selective muscarinic antagonist), oxybutynin (an M_1 and M_3 antagonist) and darifenacin (an M_3 selective antagonist) are equally effective at inhibiting bladder contraction in vivo, but that tolterodine is more potent at inhibiting contraction of the bladder than salivation in the cat. In contrast, oxybutynin and darifenacin have more effect on salivation than on bladder contraction, suggesting that a selectivity for M_3 over M_2 receptors results in a more potent effect on salivation than on bladder contraction in vivo. Interestingly, similar profiles have been demonstrated for tolterodine and AQ-RA 741(an M_2 selective antagonist); these drugs at low doses are more potent at inhibiting bladder contraction than salivation in the anesthetized cat. These results suggest that M_2 receptors have a role in bladder contraction in vivo [24]. From these in vivo data it has been suggested that it is possible to separate the effects of muscarinic antagonists on the bladder and salivary gland [24, 51]. Newgreen et al. [50] have reported that darifenacin and tolterodine have an 9 and 5 fold selectivity, respectively, for the bladder over the salivary glands in the dog in vivo. Wallis and Napier [74] have also reported that darifenacin is more effective in inhibiting salivary secretion than in reducing micturition pressure in the conscious rat.

Characterisation of presynaptic muscarinic receptor subtypes in the urinary bladder

Although the presence of M_1, M_2 and M_4 receptors has been questioned, especially in human bladder [71], the presence of M_1, M_2, M_3, and M_4 receptors has been identified by reverse transcriptase-polymerase chain reaction in the rat bladder [9]. The activation of inhibitory presynaptic M_2 or M_4 receptors and facilitatory presynaptic M_1 receptors on cholinergic terminals in urinary bladder have been identified in studies investigating [^{3}H] acetylcholine(ACh) release and contractile response to electrical field stimulation. D'Agostino et al. [17] have reported the presence of inhibitory presynaptic muscarinic receptors in rat bladder, while Somogi and deGroat [59] have characterised this inhibitory presynaptic receptor as the M_2-receptor subtype. Somogi et al. [61, 60] have also shown that continuous stimulation of postganglionic cholinergic nerves leads to the activation of presynaptic facilitatory M_1 receptors which enhance [^{3}H]ACh release in the rat, cat, and human bladder. This facilitatory mechanism is thought to be upregulated after spinal cord injury and mediated by M_3 subtype [60]. The prejunctional facilitatory M_1 receptors and inhibitory M_2 receptors regulating acetylcholine release and contractions from electric field stimulation have also been reported in rat and rabbit bladder [9, 30]. However, Alberts [2] has reported that the potency of a number of muscarinic antagonists at enhancing the secretion of [^{3}H]ACh in the guinea pig and rat bladder correlate best with values for the M_4 muscarinic receptor subtype. This suggests the prevalence of a presynaptic M_4 receptor, a muscarinic receptor subtype that is not coupled to adenylate cyclase. D'Agostino et al. [18, 19] have confirmed the role of presynaptic M_4 receptors in rat and human bladder, using new muscarinic antagonists that can distinguish between M_2 and M_4 receptors (Fig. 1).

Clinical effectiveness and muscarinic subtype selectivity of anticholinergic drugs for the treatment of overactive bladder

Propantheline bromide, emepronium bromide and trospium chloride, are quaternary ammonium compounds,

and are non-selective anti-muscarinic drugs. *Propantheline bromide and trospium chloride* also have ganglionic blocking action. Although the clinical effects of these non-selective anticholinergics have been reported [7, 13, 43], several reports have shown insufficient efficacy for treatment of urinary incontinence [55, 65, 72]. However, trospium chloride has been found to be effective in the treatment of detrusor hyperreflexia in a placebo-controlled double-blind study; it was as effective as oxybutynin but with fewer side effects [41, 63].

Recently, the tertiary amines such as oxybutynin, propiverine, and tolterodine have been commonly used for bladder overactivity.

Oxybutynin chloride has seven- to tenfold higher affinity for M_1- and M_3-receptors than for M_2-receptors [24, 51]. It has been used for the treatment of urge incontinence for more than 20 years [3] and its efficacy has been established in double-blind studies [45, 65]. It also has a direct muscle-relaxant (spasmolytic) effect, calcium antagonistic actions and local anaesthetic actions [32]. However, it has been reported that the concentrations required for its direct smooth muscle relaxant effects are greater (500 fold) than those at which its antimuscarinic effects are exerted [32]. Also the effects of oxybutynin on the bladder in vivo correlate significantly only with its antimuscarinic activity, and do not appear to be related to its direct smooth muscle relaxant effects or local anesthetic effects [52]. Thus when given systemically, oxybutynin acts mainly as an antimuscarinic drug [5]. Oxybutynin has higher affinity for muscarinic receptors in the parotid gland than the bladder in radioligand binding studies, and has more selectivity for salivation than for bladder contraction in vivo [51].

Oral oxybutynin has an extensive first-pass metabolism. Its metabolite, N-desethyl-oxybutynin is equally potent as oxybutynin in terms of antimuscarinic activity and selectivity profile in vitro [69]. The serum concentrations of N-desethyl-oxybutynin are two- to fivefold higher and persist longer than the parent compound. The affinity of N-desethyl-oxybutynin for the muscarinic receptors of the parotid gland is significantly higher than for those of the bladder [69], and it has been suggested that the adverse effects of oxybutynin may be mediated to a large extent by N-desethyl-oxybutynin [12, 57, 69]. The optimal application of oxybutynin would allow continuous absorption without variations in serum levels [57]. Thus, intravesical oxybutynin [12], oxybutynin rectal suppositories [75], vaginal inserts [57] and oxybutynin patches are considered to cause fewer side effects due to reduced plasma concentration of this metabolite. Similarly, controlled release oxybutynin chloride, a slow release formation of oxybutynin, has also been reported to have an equivalent effects on urge incontinence yet cause less dry mouth [3].

Propiverine has a dual mode of action which includes both non-selective antimuscarinic and calcium antagonistic properties. These two effects occur at similar concentrations of propiverine in rat and guinea pig bladder in vitro [67].

Tolterodine is a new drug for the treatment of overactive bladder. Neither tolterodine nor its major active 5-hydroxymethyl metabolite (5-HM) has any selectivity for muscarinic receptor subtypes [51]. Radioligand binding data has shown that tolterodine has eight times less potency than oxybutynin at the muscarinic receptor in the guinea-pig parotid gland [51]. In functional studies in vivo, tolterodine is found to be equipotent to oxybutynin, but has selectivity for the bladder over salivary glands in the cat [51] and dog [50].

The clinical efficacy of tolterodine has been verified in double-blind, placebo-controlled studies [1, 6, 20, 31, 37, 46, 54]. In urodynamic studies, an increase in maximum cystometric capacity and volume at first contraction have been reported for patients with detrusor overactivity [31, 37, 54] and detrusor hyperreflexia [68]. In a placebo-controlled study comparing tolterodine and oxybutynin, these drugs were found to be equally effective in treatment of frequency and incontinence [1]. Tolterodine at a dose of 2 mg twice daily and oxybutynin at 5 mg three times daily have been reported to be equivalent in their effectiveness [6, 20]. However, tolterodine appeared to be better tolerated than oxybutynin, particularly with regard to dry mouth, where 69–80%, 30–50% and 15–21% of patients treated with oxybutynin, tolterodine, and placebo, respectively, reported this side effect [1, 6, 20, 68]. However, cardiovascular side effects would also be anticipated for tolterodine due to its actions at M_2 receptors. Appell [6] has reported, from meta-analysis, no significant difference in the incidence of cardiovascular adverse events among individuals receiving 2 mg tolterodine (4%), 5 mg oxybutynin (6%), and placebo (8%), although 1 mg tolterodine produced a higher incidence (12%) of these events. A slight dose-dependent increase in heart rate has been reported for tolterodine [37, 54]. However, no dose-related changes in ECGs (especially QTc interval) have been demonstrated in tolterodine treated groups [6, 37, 46, 54]. Thus, this drug is considered well-tolerated and cost-effective in the treatment of overactive bladder [34].

Darifenacin has a 7-fold higher affinity for M_3-receptors than for M_2-receptors [24], but has a marginal selectivity for the bladder over the salivary gland [50, 71]. The effectiveness of this drug for detrusor instability has been reported as measured by ambulatory urodynamics [56]. Clinical trials to determine its efficacy for overactive bladder are currently being considered.

Discussion

From the experimental results we can consider that M_2 receptors are the predominant receptors present in the urinary bladder, but that M_3 receptors mainly mediate contraction. Since agents with a selective action on the bladder are considered preferable for the treatment of urinary incontinence, the selective M_3 antagonists have been thought to be useful for the treatment of this

condition. However, the side effects such as dry mouth caused by the M_3 selective agent oxybutynin are considerable because M_3 receptors also mediate contraction of the iris and intestine, and secretion of the salivary gland.

Other muscarinic receptor subtypes may also have roles for the contraction of urinary bladder. M_2 receptors modulate detrusor contraction by inhibiting the relaxation mediated by β-adrenoceptors, and they may contribute more to the contraction of the bladder in vivo or with pathological states e.g. bladder denervation or spinal cord injury.

In addition, the prejunctional inhibitory M_2 or M_4 receptors and prejunctional muscarinic M_1 receptors may be important targets for anticholinergic drugs. The role of muscarinic receptor subtypes in the central nervous system is poorly understood, and the actions of muscarinic receptor antagonists in this area should also be investigated. From this standpoint, non-selective muscarinic antagonists such as tolterodine may be more effective, with fewer of those side effects that are mediated by M_3 receptors, although non-selective antagonists may have other side effects: M_2-receptor antagonism may have effects on heart rate and M_1-receptor antagonism on gastric acid secretion and cognitive function [71]. Thus, although it is not known which antimuscarinic drugs selective for the muscarinic subtypes have better efficacy and tolerability, the non-selective antimuscarinic drugs seem to be better than M_3-selective antagonists in clinical efficacy.

However, with regard to M_3 selective antagonists, controlled release, or intravesical, intravaginal, or rectal (suppository) administration have been reported to cause fewer side effects, at least for oxybutynin. Darifenacin, a new M_3 selective antagonist has been reported to have selectivity for the bladder over the salivary gland in vivo [50, 71]. A clinical study to determine its efficacy and safety has been under consideration.

To verify which antimuscarinic drugs selective for the muscarinic subtypes have best efficacy and tolerability, comparative clinical trials between M_3 selective antagonists, such as darifenacin or controlled release oxybutynin, and non-selective compounds, such as tolterodine, are required in the future [71].

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