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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. M₂ receptors are the predominant cholinoreceptors present in urinary bladder, but mainly the minor population of M₃ receptors mediate its contraction. M₂ 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 M₂ or M₄ receptors and prejunctional facilitatory muscarinic M₁ 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 M₃-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 M₃ 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 M₃ selective antagonists and non-selective compounds, such as aloterodine, are required in the future.

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

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 parasym pathetic 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 result 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 M₁–M₅ has been recommended rather than m₁–m₅) have been cloned, and M₁–M₄
subtypes correlate well with the M₁–M₄ gene products pharmacologically [15]. M₁ receptors prevail in neuronal tissues (cerebral cortex, hippocampus, sympathetic ganglia) and are also present in glands. M₂ receptors are present in the heart, hindbrain and smooth muscle. M₃ receptors prevail in exocrine glands and are also found in smooth muscle and the brain. M₄ receptors are found in the basal forebrain and striatum. It has been reported that M₅ receptors are expressed in the substantia nigra [15]. A pharmacological correlate of the M₅ gene has been defined, but because of the similarity in pharmacological profiles of M₄ and M₅ subtypes and the current lack of a high affinity M₅ 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 M₂ and M₃ subtypes, but not the M₁, M₂, M₄ 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 M₂ and M₃ 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 M₁, M₂, M₃, and M₄ transcripts in rat bladder. At protein level using receptor binding, M₁, M₂, and M₃ receptors have been detected in human detrusor muscle [35], but other studies have detected only M₂ receptors [25] or a mixed M₂ and M₃ receptor population [47, 77]. Similarly in immunoprecipitation studies, only the M₂ and M₃ subtypes have been precipitated in rat, rabbit, guinea pig, and human [73].

Thus, a predominance of the M₂ muscarinic receptor subtype, with a minor population of M₃ 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 M₂ and M₃ 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 M₃ 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 M₃ receptors. A significant correlation has also been found with the M₅ receptors which reflects the lack of selective muscarinic antagonists which can discriminate M₃ and M₅ subtypes [16]. However, the correlation is better at M₃ than M₅ subtypes when using darifenacin and oxybutinin [58, 78], agents which display some selectivity for M₃ receptors over the M₅ subtype [21, 74]. Furthermore, the M₅ gene has not been identified in the bladder [9, 76, 73]. The predominant role of the M₃ subtype in mediating contraction of the bladder has been confirmed by the experiments using mutant mice lacking the receptor gene for the M₅ 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 (pA₂) values of muscarinic antagonists, indicating that M₃ receptors mediate the contraction of the rat urinary bladder [39].

These observations suggest that it is a minor population of M₃-receptors which mediates contraction of the detrusor muscle and that M₂ receptors are not directly involved in contraction. Muscarinic M₃-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 effect of antisemariacin drugs in the treatment of overactive bladders may lead to withdrawal of medication [1]. Thus, drugs which have more selectivity for the bladder is desirable. Because M₃ receptor subtypes have been reported to mediate salivary gland secretion, drugs selective for M₃ receptors have been considered to cause dry mouth. Indeed, oxybutynin, a selective M₃ 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 M₃ 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 M₅ receptors in the control of salivary secretion [71], although this has been disputed [21].

**M₂-muscarinic receptors**

M₂ receptors couple to the pertussis toxin-sensitive guanine nucleotide regulatory protein Gi and inhibit adenylyl cyclase activation [14, 22, 26, 64] (Fig. 1). Although