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Muscarinic Receptors and Genitourinary Smooth Muscle Function

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The biological effects resulting from parasympathetic nerve stimulation in genitourinary tissues are mediated chiefly through muscarinic cholinoceptors of which there are five known subtypes (M_1-M_5). With the exception of the corpus cavernosum, which relaxes in response to muscarinic receptor agonism, most genitourinary tissues contract, either directly or indirectly, when exposed to muscarinic receptor agonists. In recent years, the availability of selective pharmacological tools has allowed us to make considerable strides in our understanding of the functional role of individual muscarinic receptor subtype(s) in genitourinary tissues. The muscarinic receptor subtype mediating the contractile response is dependent on the species, tissue, and in some cases, region of tissue, under study. Recent studies have unmasked the functional role of M_2 receptors in the urinary bladder and these findings may set the stage for the development of safer and more efficacious subtype selective muscarinic antagonists for the treatment of overactive bladder. Unambiguous elucidation of muscarinic receptor pharmacology in the urethra, prostate, vas deferens and corpus cavernosum requires additional rigorous studies. Lastly, the identity of the muscarinic receptor mediating uterine contraction continues to generate controversy.

The biological effects resulting from parasympathetic nerve stimulation to peripheral tissues, including smooth muscles, are principally mediated through activation of muscarinic cholinoceptors[1]. The muscarinic receptor family is composed of five subtypes (M_1-M_5), encoded by five distinct but homologous intronless genes, with structural features consistent with a seven transmembrane G-protein coupled receptor[2]. In general, muscarinic M_1, M_3 and M_5 receptors preferentially couple to Gq family of guanine-nucleotide binding proteins (G-proteins) causing augmentation of phosphoinositide hydrolysis, whereas activation of muscarinic M_2 and M_4 receptors preferentially inhibit adenylyl cyclase activity via stimulation of Gi family of G-proteins[2]. Pharmacologically, the five subtypes can be discriminated by several ligands, most useful of which include pirenzipine, tripitramine, darifenacin, MT3 toxin and AQ-RA-741 (see Table 1,2,3). Since few ligands possess marked selectivity for any given subtype, operational characterization of muscarinic receptors relies upon the affinity profile of an array of antagonists.

Several tissues of the genitourinary tract are innervated by the parasympathetic nervous system and, in most of these, activation of postjunctional muscarinic receptors causes smooth muscle contraction (see Table 2). A comprehensive overview of autonomic innervation of genitourinary tissues can be found in Maggi[4]. The pharmacology of muscarinic receptors in the urinary bladder has been extensively investigated, owing to the clinical utility of muscarinic receptor antagonists in the treatment of overactive bladder. Other tissues have also been studied, one of the most complex being the guinea-pig uterus in which the existence of an atypical receptor has been suggested. By contrast, muscarinic receptors in other genitourinary tissues, including urethra, prostate and

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TABLE 1 : ANTAGONIST AFFINITY ESTIMATES (PK_B/PKi) FOR KEY LIGANDS FOR MUSCARINIC RECEPTOR SUBTYPES[2,3]

Ligand	M_1	M_2	M_3	M_4	M_5
Pirenzepine	7.8-8.5	6.3-6.7	6.7-7.1	7.1-8.1	6.2-7.1
Tripitramine	8.4-8.8	9.4-9.6	7.1-7.4	7.8-8.2	7.3-7.5
Darifenacin	7.5-7.8	7.0-7.4	8.4-8.9	7.7-8.0	8.0-8.1
MT3	7.1	<6	<6	8.7	<6
AQ-RA-741	7.5	8.4	7.2	8.2	6.1

TABLE 2 : PHARMACOLOGY OF MUSCARINIC RECEPTOR-MEDIATED RESPONSES IN SMOOTH MUSCLE FROM GENITOURINARY TISSUES

Tissue	Species	Receptor	Response
Uterus	Rat, guinea-pig	M_2 atypical	Contraction
Urinary bladder	Pig, rat, mouse, rabbit, guinea pig, man	M_3	Direct contraction
	Rat	M_2	Indirect contraction
Urethra	Rat, rabbit, dog, man	?	Contraction
Ureter	Pig	M_1	↑ phasic contractions
	Dog	?	↑ rhythmic contractions
Prostate	Rat	M_3	Contraction
	Dog	M_2	Contraction
Vas deferens	Rat	?	Contraction
	Rabbit	M_2	↑ neurogenic contractions
	Mouse	M_3	↑ neurogenic contractions
	Man	M_1	Contraction
Seminal vesicle	Guinea-pig	M_3	Contraction
Testis (capsule)	Rat	?	Contraction
Corpus cavernosum	Man, rabbit	M_3	Relaxation (NO)
	Man	M_2	?

corpus cavernosum, have been poorly characterized. This article reviews the current understanding of the pharmacology of muscarinic receptors in several major genitourinary tissues.

Uterus:

The uterine body receives an extensive cholinergic innervation[5]. Nerve stimulation-evoked contractions are completely abolished by atropine, providing evidence for cholinergic (muscarinic) neurotransmission[6]. The identity of the muscarinic receptor mediating uterine (myometrial) contraction, studied most extensively in the guinea-pig, has been the subject of immense controversy. Muscarinic M_2 receptors were originally proposed to mediate contraction of guinea-pig myometrial tissue, a conclusion based upon the preponderance of M_2 binding sites and also pA_2 values for hexamethonium, pirenzepine and methoctramine in functional studies[7]. This proposal was corroborated in subsequent studies by Bognar *et al.*[8] and Doods *et al.*[9] in which a range of antagonists were

studied. Dorje *et al.*, however, have suggested the involvement of muscarinic M_4 receptors, given the high affinity of hexahydrosiladifenidol[10]. These findings have not been confirmed and subsequent measurement of the affinities for several compounds, including himbacine[9] or imperialine[11], antagonists that discriminate muscarinic M_2 from M_4 receptors, suggest that muscarinic M_2 receptors mediate contraction. More recent studies using highly selective probes, including tripitramine and MT3 toxin, have definitively ruled out the involvement of both M_2 and M_4 receptors and have, instead, implicated the possible involvement of a muscarinic receptor with an atypical operational profile[12]. Interestingly, studies in the rat isolated uterus have yielded data which are consistent with the involvement of M_2 and, additionally, an M_3 or atypical receptor[13]. At present, the muscarinic receptor subtype mediating contraction of human myometrium has not been characterized, although given the above findings in rat and guinea-pig, this may be worthy of investigation.

Biochemical studies in guinea-pig myometrium have shown that both inhibition of adenylyl cyclase and stimulation of phosphoinositide hydrolysis occurs in response to muscarinic receptor activation, the former being pertussis toxin sensitive and attributed to muscarinic M_2 and the latter to M_3 receptor activation[14,15]. Since contraction of guinea-pig myometrium is insensitive to pertussis toxin[16] the biochemical basis for muscarinic M_2 (or an atypical receptor)-mediated contraction of guinea-pig uterus is unclear.

Urinary bladder and Urethra:

Histochemical studies of the human bladder have demonstrated a rich plexus of intramural acetylcholinesterase-positive nerves, consistent with dense cholinergic innervation[17]. Activation of the cholinergic system is the major pathway by which bladder contraction, and thus voiding, is achieved in humans and primates[18]. This may be less evident in species such as the cat or rat where excitatory innervation is partly non-cholinergic[18].

Northern blot hybridization using receptor specific probes have revealed the presence of only m_2 and m_3 mRNA in the rat and pig bladder[19,20]. In semi-quantitative polymerase chain reaction experiments, the presence of only m_2 and m_3 transcripts could be detected in the human bladder[21]. Using antisera directed against each of the five muscarinic subtypes, only m_2 and m_3 subtypes could be precipitated from rat, rabbit, guinea-pig and human bladder membranes[22,23]. Furthermore, it was shown that the m_2:m_3 ratio was 9:1 in the rat bladder and 3:1 in the bladders of other species studies[23]. In radioligand binding studies, the existence of multiple sites was originally proposed by Monferinni *et al.*[24] who demonstrated that AF-DX-116 competed for specific muscarinic binding sites labeled by [^{3}H]-N-methyl scopolamine in a heterogenous manner consistent with high affinity and low affinity sites in the ratio of approximately 9:1. More recently, competition binding studies using tripitramine, a ligand that displays >100-fold selectivity for M_2 over M_3 receptors have clearly shown a dominant (60-80%) M_2 population (high affinity for tripitramine, pKi=9.4) and a (20-40%) M_3 population (low affinity for tripitramine, pKi=7.1) in the urinary bladder of rat and man[25]. Overall, the results from radioligand binding studies closely parallel the findings from immunological studies indicating the preponderance of M_2 receptors.

Muscarinic receptor agonists induces phosphoinositide stimulation[26] and adenylyl cyclase inhibition in human bladder smooth muscle cells via receptors whose pharmacological profile equates with M_3 and M_2 receptors, respectively. Muscarinic receptors have also been shown to inhibit K_{ATP}[28] and Ca-activated K-channels[29] in bladder smooth muscle from guinea pig and rat, respectively, although the pharmacological profile of these effects was not thoroughly investigated. M_2 receptor-mediated signaling, apart from inhibition of adenylyl cyclase inhibition, such as activation of Rho proteins[30] and opening of non-selective cation channels[31], have been demonstrated in other smooth muscles and may also be operative in the bladder (see Fig. 1).

Pharmacological antagonist characterization of muscarinic receptors mediating direct contraction of detrusor in rat[32,33], rabbit[34], mouse[35], guinea-pig[36], monkey[37] and human[38] bladder suggests the singular involvement of M_3 receptors. This raises questions about the functional role of the M_2 receptors, which predominates in every species studied. Emerging evidence, however, appears to shed light on the role of M_2 receptors in the bladder. Under conditions in which M_3 receptors are selectively alkylated, M_2 receptors were shown to reverse β-adrenoceptor-mediated relaxation (referred to as indirect contraction or re-contraction)[33,39]. These findings suggest that the role of M_2 receptors in the bladder may be to oppose β-adrenoceptor-mediated detrusor relaxation, which contributes to urine storage. It can therefore be

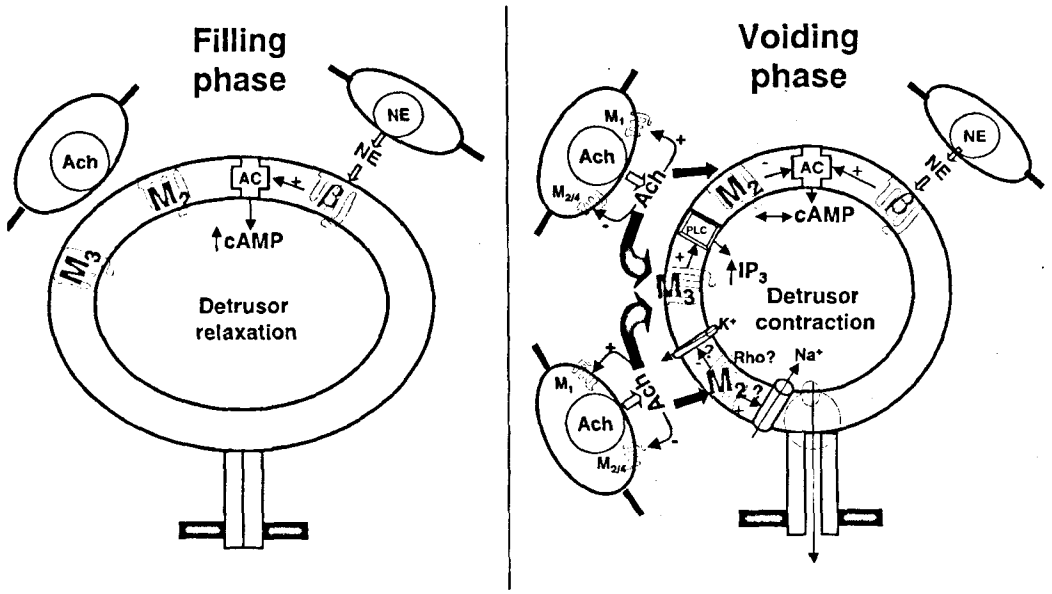

Fig. 1 : A schematic diagram summarizing proposed mechanisms underlying the stimulatory effects of parasympathetic nervous system on the voiding process. During the filling phase, the sympathoinhibitory drive to the bladder is tonically active and norepinephrine (NE) increases the compliance of the bladder through β-adrenoceptor-mediated relaxation of the detrusor (via stimulation of adenylyl cyclase (AC)). The parasympathetic drive to the bladder, which is normally suppressed during the filling phase, is augmented during the voiding phase. Acetylcholine (Ach), released from post-ganglionic cholinergic nerves interacts with post-junctional M_3 muscarinic receptors to cause direct detrusor contraction (via phospholipase C (PLC) stimulation) whereas activation of postjunctional M_2 muscarinic receptors inhibits β-adrenoceptor-mediated stimulation of AC thereby causing reversal of the relaxant tone to the bladder. This dual mechanism causes more efficient and complete voiding of urine. Other postulated M_2 contractile mechanisms, demonstrated in other smooth muscles and which could also be operative in the bladder, include inhibition of K channels, activation of non-specific cation channels and stimulation of Rho proteins. The magnitude of the postjunctional response is also determined by the extent to which pre-junctional inhibitory (M_2/M_4) and facilitatory (M_1) muscarinic receptors modulate Ach release.

postulated that, during bladder voiding, M_3 receptors cause direct smooth muscle contraction, whereas, M_2 receptors reverse sympathetically mediated relaxation and these two effects synergise to cause more efficient discharge of urine (Fig. 1).

In patients with various forms of bladder overactivity, it is thought that excessive cholinergic input accompanied by muscarinic receptor supersensitivity contributes to generation of involuntary detrusor contractions leading to urinary incontinence[40]. This underlies the established usefulness of non-selective muscarinic receptor antagonists, such as oxybutynin and tolterodine, in the treatment of overactive bladder[41]. From a therapeutic standpoint, combined blockade of both M_2 and M_3 receptors may seem, intuitively, to be the logical approach. However, if either M_2 or M_3 receptors assumes a greater pathophysiological role in disease states, then selective blockade of one of these receptors may be the more rationale approach. Interestingly, a recent intriguing finding, which requires confirmation, is that direct contractile receptors are mediated by M_2 receptors in the urinary bladder isolated from rats which previously underwent pelvic denervation or spinal cord injury, as opposed to the involvement of M_3 receptors in the bladders of control rats[42]. This finding may argue for the development of selective M_2 receptor antagonists for the treatment of overactive bladder.

The bladder neck and proximal urethra are also innervated by the parasympathetic system although muscarinic receptor pharmacology in these regions has been less well studied in comparison to the bladder body. A recent study in the rabbit urethra has demonstrated the

predominance of M_2 sites in the tissue although functional characterization of the contractile response indicated the involvement a mixed receptor population[43].

Ureter:

The ureteral smooth muscle functions to transport urine from the kidneys to the urinary bladder, by induction of peristalsis. Histochemical studies have demonstrated a rich cholinergic innervation of the intravesical ureter but not the proximal ureter[44]. Cholinergic nerves are present in all three layers of the ureter, including the outer adventitia, middle smooth muscle layer and the inner mucosal layer[45], although the function of acetylcholine release in these layers is unclear. In the pig isolated intravesical ureter, carbachol increases the frequency of phasic contractile activity via activation of multiple muscarinic receptors and enhances the basal tone via stimulation of muscarinic M_1 receptors[46]. Radioligand binding in the pig intravesical ureter have shown a predominance of M_2 receptors[47]. Morita *et al.*, have reported that muscarinic agonists, such as carbachol, augment the occurrence of rhythmic contractions in canine ureter[48]. The muscarinic receptor subtype mediating this response has not been investigated.

Prostate:

The human prostate is sparsely innervated by cholinergic nerves[49]. Acetylcholinesterase-positive fibres can be found in the fibromuscular stroma, around the acini and ducts of prostate glands and along blood vessels[49]. Studies in rat and guinea-pig prostatic tissues have demonstrated a minor role of acetylcholine in neurogenic contractile responses[50]. The majority of muscarinic receptors in the human prostate are of the M_1 subtype but these are localized to the glandular epithelium[51]. However, muscarinic receptor agonists can cause contraction of isolated smooth muscle strips from human prostatic capsule[52]. Indeed, muscarinic M_2 receptor binding sites can be detected in primary cultures from human prostatic smooth muscles[53]. In rat and human prostate, activation of muscarinic receptors inhibits adenylyl cyclase activity elevated by both forskolin and/or β–adrenoceptor agonists[53,54]. The effect, if any, of muscarinic receptors, M_3 or otherwise, on activation on phosphoinositide hydrolysis in these cells is unknown. Pharmacological studies in rat[55] and dog[56] isolated prostate have revealed that the pharmacological profile of muscarinic receptors mediating contractile responses in this tissue equates with that of M_3 and M_2 receptors, respectively. Similar operational studies in human prostatic tissue have never been undertaken to date.

Vas deferens, seminal vesicle, testis and epididymis:

Muscarinic agonists cause contraction of vas deferens from several species, although the muscarinic receptor subtype involved varies with species. In the dog, the density of muscarinic receptors is highest in the prostatic portion and lowest in the epididymal portion, possibly reflecting different levels of parasympathetic innervation[57]. In the rat vas deferens, Doggrell[58] originally suggested that contractions of the epididymal portion are mediated by M_1 and M_2 muscarinic receptors whereas a recent study has implicated the involvement of M_3 receptors in contraction of the whole vas deferens[59]. In the human vas deferens, muscarinic M_1 receptors mediate contractile responses to exogenous acetylcholine[60]. In the rabbit vas deferens, muscarinic M_2 receptors mediate potentiation of neurogenic contractions[61], whereas muscarinic M_3 or M_4 receptors mediate a similar effect in the mouse vas deferens[62].

Cholinergic innervation in the seminal vesicle is restricted largely to the epithelium, with the smooth muscle receiving little or no innervation[49]. However, Alzuhair *et al.* have reported that a rich cholinergic plexus is present in the innercircular muscle layer in guinea-pig seminal vesicle[63]. Contractions of this tissue are mediated by activation of muscarinic M_3 receptors and northern blot studies have failed to find evidence for expression of muscarinic M_2 mRNAs[64].

Autonomic innervation plays only a minor role in the control of the testis and cholinergic innervation of this tissue is sparse or absent[65]. Muscarinic Receptors may mediate contraction of the smooth muscle capsule although the subtype is undefined. In regards to the epididymis, the extent of cholinergic innervation varies according to species and location[65].

Corpus cavernosum:

The parasympathetic nervous system plays an important role in tumescence and penile erection by contributing to relaxation of corpus cavernosal smooth muscle although these effects are mediated via a non-cholinergic, non-adrenergic transmitter, namely nitric oxide[66]. Exogenous acetylcholine does, however, cause relaxation of precontracted human and rabbit corpus caverno-

sum via muscarinic receptor mediated release of nitric oxide from endothelial cells[67,68]. Furthermore, intracavernous injection of acetylcholine in adult male dogs produces increases in intracavernous pressure accompanied by sustained erection[69]. Radioligand binding studies have shown the presence of muscarinic M_3 receptors on endothelial cells derived from the human corpus cavernosum, perhaps suggesting the involvement of this receptor in relaxation of smooth muscle via a nitric oxide/c-GMP pathway[70]. *In situ* hybridization studies have, additionally, shown the presence of muscarinic M_2 receptor mRNA in smooth muscle cells of the human corpus cavernosum[71], although the precise role of muscarinic M_2 receptors in direct modulation of smooth muscle tone is unclear.

Summary:

Smooth muscles from most genitourinary tissues, with the exception of corpus cavernosum, contract in response to muscarinic receptor stimulation (see Table 2). The contractile response could be either direct or indirect (i.e., modulation of neurogenic contractions, as in vas deferens, or reversal of pre-existing relaxant tone, as in the urinary bladder). The recent availability of potent and selective antagonists, such as darifenacin and tripitramine, has immensely aided the operational characterization of muscarinic receptors in genitourinary tissues. The muscarinic receptor subtype mediating the contractile response is dependent on the species, tissue and in some cases, region of tissue, under study. Clearly, density of receptor protein or mRNA transcripts in a given tissue does not predict the relative contribution of a gives subtype towards the functional response. Emerging findings on the functional role of M_2 receptors in the urinary bladder, especially in disease states, may trigger the development of safer and more efficacious subtype selective muscarinic antagonists for the treatment of overactive bladder. The identity of the muscarinic receptor mediating uterine contraction continues to elude pharmacologists. Lastly, definitive elucidation of muscarinic receptor pharmacology in the urethra, prostate, vas deferens and corpus cavernosum requires further and more rigorous, studies.

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