Invited Review

Muscarinic receptor subtypes of the bladder and gastrointestinal tract

Toshimitsu UCHIYAMA[1] and Russell CHESS-WILLIAMS[2]

[1]*Department of Pharmacology, Faculty of Medicine, Toho University, Ohta-ku, Tokyo 143-8580, Japan*

[2]*Department of Biomedical Science, University of Sheffield, Sheffield, UK*

Abstract

The parasympathetic nervous system is responsible for maintaining normal intestinal and bladder function, contracting the smooth muscle by releasing the neurotransmitters acetylcholine (ACh) and ATP and relaxing sphincters by releasing nitric oxide. ACh is the main transmitter released and smooth muscle contraction is mediated via a mixed M2/M3 receptor population; M3 receptors acting via phospholipase C and M2 receptors acting via inhibition of adenylate cyclase. In ileal, colonic, gastric and bladder (detrusor) smooth muscle the density of M2 receptors is far greater than the density of M3 receptors, the M2:M3 ratio being 3:1 in most species including man. Despite the predominance of M2-receptors, direct contraction of intestinal and detrusor smooth muscle is mediated via the M3-receptor subtype and only this subtype is involved in contraction *in vitro*. Furthermore, knocking out the M3-receptor gene can have severe consequences on intestinal and bladder responses. In some tissues however M2-receptors may mediate an indirect "re-contraction" whereby a reduction in adenylate cyclase activity reverses the relaxation induced by β-adrenoceptor stimulation. Thus, intestinal and bladder responses to muscarinic agonists are slightly depressed in M2 receptor knockout mice. The role of receptor subtypes in disease is unclear, but an enhancement of M2 receptor mediated responses has been reported to occur in diabetes. Animal models suggest that M2 receptors may play a greater role in some situations such as in the denervated bladder and intestine. In human disease the mechanisms operating are not so clear. Detrusor sensitivity to muscarinic agonists is enhanced in the neurogenic overactive bladder, but there is controversy surrounding the role of M2 receptors and conflicting results have been reported. Thus, the main muscarinic receptor mediating contraction in normal smooth muscle is the M3 receptor, but M2 receptors are also present and possibly may have an enhanced role in disease.

Key words: detrusor, bladder, intestinal, muscarinic receptors

Introduction

The lower urinary tract and the gastrointestinal tract receive a dense nervous innervation

Correspondence to: Dr. R. Chess-Williams, Department of Biomedical Science, University of Sheffield, Western Bank, Sheffield S10 2TN, UK
Phone: +44-114-2224680 Fax: +44-114-2765413 e-mail: R.Chess@sheffield.ac.uk

from the lumbosacral parasympathetic outflow and it is this system that exerts the major influence on activity. A sympathetic innervation also exists, and interactions between the systems operate at a prejunctional and postjunctional level. The main neurotransmitter released within the parasympathetic system is acetylcholine (ACh), but non-adrenergic, non-cholinergic transmitters are also co-released along with Ach (Burnstock, 2001a; 2001b). Thus ATP, nitric oxide and peptides such as VIP are often co-released with Ach. However the main transmitter remains ACh and the receptors mediating postganglionic responses are the muscarinic receptors.

Although not located on smooth muscle, muscarinic receptors at other sites in the bladder and gastrointestinal tract also influence smooth muscle contraction in these systems. In both the lower urinary tract and the gastrointestinal tract muscarinic receptors are found prejunctionally where they influence the release of neurotransmitter from autonomic nerves. Thus, prejuctional M1 receptors enhancing Ach release have been identified in the bladder of several species (for review see Somogi and de Groat, 1999) and also in the gastrointestinal tract (Kortezova *et al.*, 1998). M2 receptors and/or possibly M4 receptors are also found prejunctionally in the bladder (Somogi and de Groat, 1999) and the rat anoccocygeus muscle (Lambrecht *et al.*, 1999), where they inhibit transmitter release. In the bladder, when nerves are stimulated at physiological frequencies, muscarinic receptor stimulation results in enhanced smooth muscle responses, the enhancement being susceptible to blockade by pirenzipine suggesting that it is the prejunctional M1 receptors that predominate under normal physiological conditions (Somogo *et al.*, 1994).

Muscarinic receptors are also found on the urothelium lining the bladder where their activation results in the release of an unidentified factor that inhibits contraction of the underlying smooth muscle (Hawthorn *et al.*, 2000). A similar situation exists in the rat bladder, where muscarinic receptor stimulation causes the release of an unidentified relaxing factor that can inhibit smooth muscle contraction (Fovaeus *et al.*, 1999). However the factor in the rat is released from the detrusor smooth muscle itself and not the urothelium. Thus muscarinic receptors are involved in regulating smooth muscle contraction at several levels within the lower urinary and gastrointestinal tracts.

Muscarinic Receptor Subtypes and Second Messenger Systems

Five muscarinic receptor subtypes have been cloned and defined pharmacologically. The pharmacology of the cloned receptors correlates well with the corresponding native receptors and both have been given the same nomenclature (M1-M5) (Caulfield and Birdsall, 1998). M1, M3 and M5 receptors couple to Gq/11 guanine nucleotide binding proteins and alter cellular activity by stimulating phospholipase C and generating the second messengers inositol trisphosphate (IP3) which induces the release of calcium from intracellular stores and diacylglycerol (DAG) which causes the influx of extracellular calcium to induce responses (Caulfield and Birdsall, 1998). M2 and M4 receptors couple to Gi proteins to induce responses via an inhibition of adenylate cyclase (Peralta *et al.*, 1988), a reduction in cAMP levels and smooth muscle relaxation. A number of other putative pathways may also be activated following

M2 receptor stimulation. These include the activation of a non-selective cation channel (Bolton and Zholos, 1997), an inhibition of potassium channels (K_{ATP} and BK) and the activation of rho kinase leading to sensitization of the contractile machinery to calcium (Togashi *et al.*, 1998).

Muscarinic Receptor Subtypes in Bladder and Gastrointestinal Smooth Muscle

Which receptor subtypes are involved in mediating contraction of the lower urinary and gastrointestinal tracts has received significant attention in recent years because of the obvious therapeutic potential of selective muscarinic antagonists in the treatment of the overactive bladder and irritable bowel syndrome. In bladder and gastrointestinal smooth muscle, both the M2 and M3 receptors are present at the mRNA and protein level. Thus Northern blot analysis and RT-PCR studies have identified mRNA for M2 and M3 in the bladder of rats (Maeda *et al.*, 1988; Mimata *et al.*, 1997), pig (Maeda *et al.*, 1988) and human (Yamaguchi *et al.*, 1996). In human bladder (Yamaguchi *et al.*, 1996), pig bladder (Maeda *et al.*, 1988) and rat gastric smooth muscle (Lin *et al.*, 1997) only the M2- and M3 receptor transcripts appear to be present. Furthermore, receptor immunoprecipitation studies have identified the presence of M2 and M3 receptor protein, but not the M1, M4 or M5-receptor protein in rat, guinea-pig, rabbit and human bladder (Wang *et al.*, 1995). Some of the early radioligand binding studies reported the exclusive presence of M2 receptors in the bladder of various species, but more recent findings with more selective drugs have confirmed the presence of both M2 and M3 receptor subtypes, although the density of M2 receptors is usually far greater than that of the M3 subtype. Thus M2 receptors outnumber M3 receptors by a factor of 3 in the bladders of most species but may be as high as 9 in some species like the rat (Wang *et al.*, 1995). A similar situation has been found for gastrointestinal smooth muscle where M2 receptors have been shown to be present in greater density than M3 in the guinea pig ileum (Michel and Whiting, 1988) and canine colon (Zhang and Buxton, 1991).

Receptor Subtypes Mediating DIRECT Contraction

The *in vitro* responses of isolated strips of gastrointestinal or lower urinary tract tissue following stimulation with agonists such as carbachol or oxotremorine have been examined in many species including humans. In all species so far examined, the responses of isolated tissue preparations appear to be mediated exclusively via the M3-muscarinic receptor subtype, with M3-receptor selective antagonists such as 4-DAMP having a high affinity for the receptor whilst M2-receptor selective antagonists like methoctramine having a relatively low affinity. Furthermore, wherever Schild analysis has been performed the plots for these antagonists have slopes of unity indicating that the M3 receptor is the only muscarinic subtype involved in mediating contractile responses to muscarinic agonists *in vitro*. Thus, direct smooth muscle contraction is mediated via the M3 receptor subtype in the cat oesophagus (Preiksaitis and Laurier, 1998), rat stomach (Lin *et al.*, 1997), guinea pig and dog ileum (Honda *et al.*, 1993; Shi and Sarna, 1997), guinea pig common bile duct (Karahan *et al.*, 1991), guinea pig colon (Ehlert, 1999), guinea pig taenia caeci (Shen and Mitchelson, 1998) and rat anoccocygeus muscle

(Weiser *et al.*, 1997). Similarly, in the lower urinary tract, responses of the bladder have been shown to be mediated via M3 receptors in all species so far examined (for review see Chess-Williams, 2002) including the human bladder (Chess-Williams *et al.*, 2002; Fetscher *et al.*, 2002).

In several studies, correlation plots have also been constructed where the affinities of a range of antagonists for the intestinal or detrusor smooth muscle have been plotted against affinities for the same antagonists obtained in radioligand binding studies with the five cloned receptor subtypes. In both the intestinal (Ehlert *et al.*, 1997) and detrusor muscle (Sellers *et al.*, 2000; Chess-Williams, 2002) the best correlations have been obtained against the cloned M3 receptor confirming that this receptor, which comprises the minority of the muscarinic receptor population in these tissues, is responsible for mediating direct contraction.

The dominant role of the M3 receptor subtype has been confirmed in second messenger studies of smooth muscle and muscarinic receptor induced increases in PI turnover have been noted in guinea-pig, rat and human bladder (Noronha-Blob *et al.*, 1989; Andersson *et al.*, 1991; Mimata *et al.*, 1997) and the rat ileum (Cabdell *et al.*, 1990) and canine colon (Zhang and Buxton, 1991). Furthermore, the receptor mediating these responses has the pharmacological characteristics of the M3 receptor subtype, and M3-antagonists such as 4-DAMP block the second messenger response with a high affinity (Harriss *et al.*, 1995). However M2 receptors are present and can be shown to be functional at a second messenger level where both an increase in phosphoinositide turnover (M3) and a decrease in cyclic AMP levels (M2) have been recorded in human detrusor smooth muscle following stimulation with carbachol (Harriss *et al.*, 1995). An interesting finding is that the contribution of M2 receptors to contraction may be greater in some regions than others. This can be seen in the lower urinary tract of the guinea pig (Wheeler *et al.*, 1995), where carbachol increases IP_3 levels in the ureter (M3), increases IP_3 levels and inhibits cAMP formation in the bladder (M3>M2), while in the urethra a decrease in cAMP is observed before an increase in IP_3 formation (M2>M3). An inhibition of cAMP accumulation by muscarinic agonists has also been noted in a number of species including the rabbit, guinea pig and human bladder (Noronha-Blob *et al.*, 1989), rat ileum (Candel *et al.*, 1990), canine colon (Zhang and Buxton, 1991), guinea-pig and rat intestine (Griffin and Ehlert, 1992; Ostrom and Ehlett, 1998). Furthermore the inhibitory effect of muscarinic agonists on adenylate cyclase can be blocked by antagonists with a high affinity for the M2-receptor.

In some tissues, stimulation of M2 receptors has also been linked to additional transduction mechanisms (Fig. 1). These include an activation of a non-selective cation channel resulting in depolarization and calcium influx in the guinea pig ileum (Inoet *et al.*, 1994; Bolton and Zholos, 1997) and an inhibition of potassium channels in canine colon (Cole *et al.*, 1989) and gastric smooth muscle (Sims *et al.*, 1990). The involvement of these mechanisms may not be simple and some of the transduction mechanisms may influence each other. Thus a rise in calcium will facilitate but not activate the non-selective cation channel resulting in a transduction process that not only requires M2 receptor stimulation but also a simultaneous rise in intracellular calcium such as that which occurs during M3 receptor activation (Wang *et al.*, 1997a; Wang *et al.*, 1997b). This would explain why most tissues have a mixed M2/M3 receptor population.

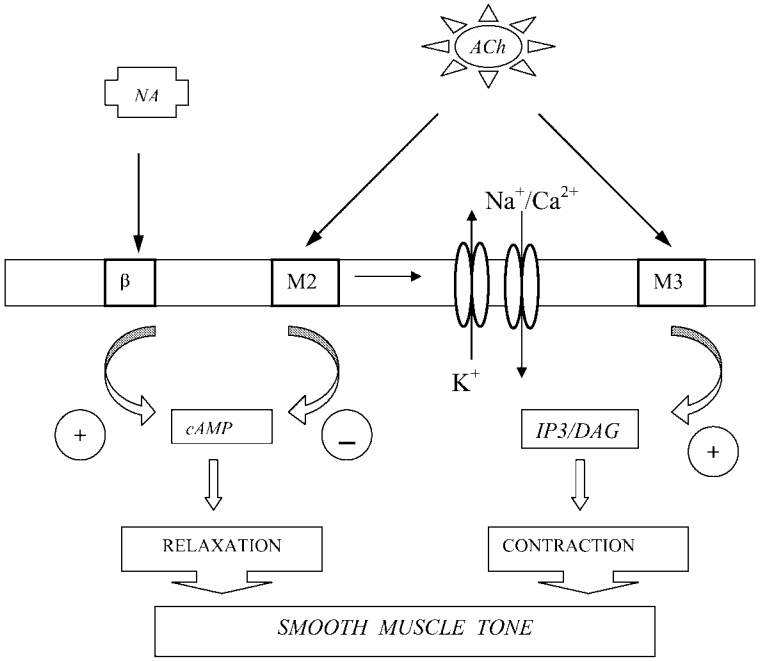

Fig. 1. Acetylcholine (ACh) stimulates M3-receptors to cause direct smooth muscle contraction via the second messengers inositol trisphostphate (IP_3) and diacylglycerol (DAG). Acetylcholine via M2 receptors also induces a contraction indirectly by inhibiting the production of cyclic AMP and reversing relaxation. In some tissues, M2 receptors may also inhibit potassium channels and open non-specific cation channels to induced contraction.

Gene Knock Out Studies

The relative importance of M2 and M3 receptors in smooth muscle function has been investigated with mice in which the gene for one or both of these muscarinic receptors is absent. In M3 knock out mice (Matsui *et al.*, 2000), severe defects were observed in smooth muscle function, with bladder contraction to carbachol being reduced by 95% and contraction of the ileum by 75%. In both tissues the remaining responses were antagonised by methoctramine, indicating a small M2 receptor component. Severe urinary retention occurred in male knock-out mice, but no gastrointestinal dysfunction *in vitro* was observed. These data suggest that smooth muscle contraction is predominantly mediated via M3 receptors but that M2 receptors also play a minor role at least in M3 knock-out animals.

In M2 knock-out mice (Stengel *et al.*, 2000), as expected cardiac responses to carbachol were abolished. In the ileum and bladders of these animals, responses to the agonist were depressed and the affinity of the M2 receptor-selective antagonist AF-DX116 was reduced. However, these effects were small (only 2-fold change in carbachol EC_{50} value and antagonist affinity) suggesting a role for M2 receptors in bladder contraction in the mouse, but not a predominant one. This has been confirmed in cystometric studies in receptor knock-out mice

where the M3 receptor was found to be the dominant receptor *in vivo* (Igawa *et al.*, 2005).

In mice in which both the M3 and M2 receptor gene have been knocked out (Matsui *et al.*, 2002), responses of the bladder and ileum to muscarinic agonists were completely abolished. Bladders were distended in males but there was no apparent dysfunction of the gastrointestinal system. Since gastrointestinal responses induced by electrical field stimulation were increased at the same time as responses to muscarinic agonists were abolished, it appears that non-cholinergic mechanisms of contraction are upregulated to maintain gastrointestinal function. A similar situation where non-cholinergic mechanisms upregulate to compensate for depressed cholinergic mechanisms has also been proposed for the bladder (Igawa *et al.*, 2005).

Pharmacological Manipulations of Tissue Receptor Populations

In several tissues including the guinea pig ileum (Ehlert *et al.*, 1995), colon (Ehlert, 1999), oesophagus (Eglen *et al.*, 1996), and the bladders of pigs (Yamanishi *et al.*, 2002) and guinea pig (Eglen *et al.*, 1994; Hedge *et al.*, 1997), the role of M2 receptors has been investigated following manipulation of the muscarinic receptor population and second messenger levels. In these tissues, removal of the M3-population with the alkylating agent 4-DAMP mustard (during M2-protection with methoctramine) and elevation of cAMP levels with isoprenaline elicits a response to muscarinic agonists that is at least partially mediated via the M2-receptor subtype (Hedge *et al.*, 1997). This is in contrast to the normal situation where responses are mediated solely via the M3-subtype.

These experiments suggest that M_2-receptors may regulate smooth muscle tone under conditions of high sympathetic activity or where M_3-receptors are dysfunctional (Eglen *et al.*, 1994). In the body of the urinary bladder the predominant effect of sympathetic nerve stimulation is relaxation mediated via β-adrenoceptors and this is thought to facilitate the urine storage phase by relaxing the detrusor smooth muscle. Activation of M_2 receptors during cholinergic activation may "switch off" sympathetic inhibitory (β) mechanisms and result in more efficient emptying of the bladder and food transit in the intestine.

Muscarinic Receptors in Disease

The sensitivity of the detrusor muscle to muscarinic agonists is enhanced in several diseased states including diabetes mellitus (Mimata *et al.*, 1995; Kanda *et al.*, 1997), denervation (Braverman *et al.*, 1998), bladder outflow obstruction (Speakman *et al.*, 1987) and ageing (Yu *et al.*, 1997). There may also be changes in receptor subtype function in disease. In the rat bladder M2 receptor density is increased in several disease models (denervation, diabetes) and M2 receptors may contribute significantly to contraction in animal models of disease but few studies have been performed in human.

Diabetes mellitus

Smooth muscle responses to muscarinic agonists are increased in diabetes and two mechanisms appear to be operating. Firstly there is an increase in muscarinic receptor density

in diabetic rat bladder (Saito *et al.*, 1997; Kanda *et al.*, 1997) and ureter (Hernandez *et al.*, 1995), which results in increased IP_3 production and a supersensitivity to agonists (Kanda *et al.*, 1997). The second mechanism is an increase in smooth muscle sensitivity to calcium (Waring and Wendt, 2000) and this may be responsible for the increase in maximum responses observed to carbachol, potassium and electrical field stimulation (Waring and Wendt, 2000). Both mechanisms operate in detrusor smooth muscle to increase tissue sensitivity and responsiveness, but in the rat ileum only the increase in responsiveness is observed in diabetic rats (Carrier and Aronstam, 1990) suggesting that only the change in calcium sensitivity occurs in the gastrointestinal tract. Northern blot analysis has demonstrated that the increase in total muscarinic receptor density is due to a selective 70% increase in the population of M2-receptors in 2-week diabetic rats (Tong *et al.*, 1999) suggesting a possible enhanced role for this receptor subtype in diabetes.

Denenervation

Denervation of the rat intestine (Osinski and Bass, 1994) or rat bladder (Braverman *et al.*, 1999) results in a supersensitivity of muscarinic responses, which have been shown to be associated with an increase in receptor density. Furthermore in both systems the affinity of M2 antagonists is increased following denervation suggesting a possible increase in role for this receptor subtype in disease.

Enhanced responses to muscarinic receptor stimulation have also been reported in the human neurogenic overactive bladder (Martin *et al.*, 1997). In the rat bladder M2-receptor density is increased by 60% following denervation and M2 receptors contribute significantly to contraction in this animal model (Braverman *et al.*, 1998). Such a change in receptor function is not observed in the rat obstructed bladder where responses continue to be mediated via the M3-receptor subtype (Krichevsky *et al.*, 1999), demonstrating the disease specific nature of these receptor changes. Increases in detrusor muscarinic M2-receptor function and M2-receptor density have also been observed in the rat following pelvic ganglion removal (Braverman *et al.*, 1998), but the physiological relevance of such changes in M2 receptor are unclear. In human bladder the role of M2 receptors in disease is controversial. In patients with neurogenic overactive bladders, Pontari *et al.* (2004) have suggested that M2 receptor function is enhanced, but we have been unable to support this finding. In detrusor strips obtained from patients with neurogenic overactive bladders due to spinal injury we have found that the detrusor smooth muscle is supersensitive to muscarinic agonists. However the antagonist affinity values for the M2-selective antagonist methoctramine were similar to the values obtained in normal tissue suggesting no change in M2-receptor function (Stevens *et al.*, 2004) and a role for M2 receptors in human disease has yet to be firmly established.

In conclusion, the role played by muscarinic receptors in the lower urinary and gastrointestinal tracts appear to be similar in the two systems. Direct contraction responses are mediated exclusively via the M3 subtype, despite the main receptor protein in these tissues being the M2 receptor subtype. M2 receptors inhibit adenylate cyclase-mediated relaxation and these receptors may be responsible for switching off sympathetically induced inhibition of smooth muscle contraction during activation of the parasympathetic nervous system. In animal

models of disease, M2 receptors appear to have an enhanced role although whether this applies to human disease requires further investigation.

References

Andersson, K.-E., Holmquist, F., Fovaeus, M., Hedlund, H. and Sundler, R. (1991). Muscarinic receptor stimulation of phosphoinositide hydrolysis in the human isolated urinary bladder. *J. Urol.* **146**: 1156–1159.

Bolton, T.B. and Zholos, A.V. (1997). Activation of M2 muscarinic receptors in guinea-pig ileum opens cationic channels modulated by M3 muscarinic receptors. *Life Sci.* **60**: 1121–1128.

Braverman, A., Legos, J., Young, W., Luthin, G. and Ruggieri, M. (1999). M2 receptors in genito-urinary smooth muscle pathology. *Life Sci.* **64**: 429–436.

Braverman, A.S., Luthin, G.R. and Ruggieri, M.R. (1998). M_2 muscarinic receptor contributes to contraction of the denervated rat urinary bladder. *Am. J. Physiol.* **275**: R1654–R1660.

Burnstock, G. (2001a). Purinergic signalling in lower urinary tract. In: Purinergic and Pyrimidinergic Signalling I: Molecular, Nervous and Urogenitary System Function, ed. by M.P. Abbracchio, and M. Williams, Springer-Verlag, Berlin Heidelberg, Chapter 15, pp. 421–515.

Burnstock, G. (2001b). Purinergic signalling in gut. In: Purinergic and Pyrimidinergic Signalling I: Molecular, Nervous and Urogenitary System Function, ed. by M.P. Abbracchio and M. Williams, Springer-Verlag, Berlin Heidelberg, Chapter 21, pp. 141–238.

Candell, L.M., Yun, S.H., Tran, L.L. and Ehlert, F.J. (1990). Differential coupling of subtypes of the muscarinic receptor to adenylate cyclase and phosphoinositide hydrolysis in the longitudinal muscle of the rat ileum. *Mol. Pharmacol.* **38**: 689–697.

Carrier, G.O. and Aronstam, R.S. (1990). Increased muscarinic responsiveness and decreased muscarinic receptor content in ileal smooth muscle in diabetes. *J. Pharmacol. Exp. Ther.* **254**: 445–449.

Caulfield, M.P. and Birdsall, N.J.M. (1998). International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol. Rev.* **50**: 279–290.

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

Chess-Williams, R., Chapple, C.R., Yamanishi, T. and Sellers, D.J. (2002). The minor population of M3-receptors mediate contraction of human detrusor muscle in vitro. J. Auton. Pharmacol. **21**: 1–6.

Cole, W.C., Carl, A. and Sanders, K.M. (1989). Muscarinic suppression of Ca^{2+}- dependent K current in colonic smooth muscle. *Am. J. Physiol.* **257**: C481–C487.

Eglen, R.M., Peelle, B., Pulido-Rios, M.T. and Leung, E. (1996). Functional interactions between muscarinic M_2 receptors and 5-hydroxytryptamine $(5\text{-HT})_4$ receptors and β_3- adrenoceptors in isolated oesophageal muscularis mucosae of the rat. *Br. J. Pharmacol.* **119**: 595–601.

Eglen, R.M., Reddy, H., Watson, N. and Challis, R.A.J. (1994). Muscarinic acetylcholine receptor subtypes in smooth muscle. *Trends. Pharmacol. Sci.* **15**: 114–119.

Ehlert, F.J. (1999). Muscarinic M3 receptor inactivation reveals a pertussis toxin-sensitive contractile response in the guinea pig colon: evidence for M2/M3 receptor interactions. *J. Pharmacol Exp. Ther.* **289**: 464–476.

Ehlert, F.J. and Thomas, E.A. (1995). Functional role of M2 muscarinic receptors in the guinea pig ileum. *Life Sci.* **56**: 965–971.

Ehlert, F.J., Thomas, E.A., Gerstin, E.H. and Griffin, M.T. (1997). Muscarinic receptors and gastrointestinal smooth muscle. In: ed. by R.M. Eglen, Muscarinic Receptor Subtypes in Smooth Muscle. CRC Press, Boca Raton, pp. 92–147.

Fetscher, C., Fleichman, M., Schmidt, M., Krege, S. and Michel, M.C. (2002). M3 muscarinic receptors

mediate contraction of human urinary bladder. *Br. J. Pharmacol.* **136**: 641–643.

Fovaeus, M., Fujiwara, M., Hogestatt, E.D., Persson, K. and Andersson, K-E. (1999). A non-nitrergic smooth muscle relaxant factor released from rat urinary bladder by muscarinic receptor stimulation. *J. Urol.* **161**: 649–655.

Griffin, M.T. and Ehlert, F.J. (1992). Specific inhibition of isoproterenol- stimulated cyclic AMP accumulation by M2 muscarinic receptors in rat intestinal smooth muscle. *J. Pharmacol. Exp. Ther.* **263**: 221–225.

Harriss, D.R., Marsh, K.A., Birmingham, A.T. and Hill, S.J. (1995). Expression of muscarinic M3-receptors coupled to inositol phospholipid hydrolysis in human detrusor cultured smooth muscle cells. *J. Urol.* **154**: 1241–1245.

Hawthorn, M.H., Chapple, C.R., Cock, M. and Chess-Williams, R. (2000). Urothelium-derived inhibitory factor(s) influence detrusor muscle contractility in vitro. *Br. J. Pharmacol.* **129**: 416–419.

Hedge, S.S., Choppin, A., Bonhaus, D., Briaud, S., Loeb, M., Moy, T.M., Loury, D. and Eglen, R.M. (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.

Hernandez, M., Garcia-Sacristan, A. and Orensanz, L.M. (1995). Muscarinic binding sites of the pig intravesical ureter. *J. Auton. Pharmacol.* **15**: 351–359.

Honda, K., Takano, Y. and Kamiya, H. (1993). Pharmacological profiles of muscarinic receptors in the longitudinal smooth muscle of guinea pig ileum. *Jpn. J. Pharmacol.* **62**: 43–47.

Igawa, Y., Zhang, X., Nishizawa, O., Umeda, M., Iwata, A., Taketo, M.M., Manabe, T., Matsui, M. and Andersson, K-E. Cystometric findings in mice lacking muscarinic M2 and M2 receptors. *J. Urol.* (in press).

Inoue, R., Waniishi, I., Yamada, K. and Ito, Y. (1994). A possible role of tyrosine kinases in the regulation of muscarinic receptor activated cation channels in guinea-pig ileum. *Biochem. Biophys. Res. Commun.* **203**: 1392–1397.

Kanda, M., Eto, K., Tanabe, N., Sugiyama, A., Hashimoto, K. and Ueno, A. (1997). Effects of ONO-2235, an aldose reductase inhibitor, on muscarinic receptors and contractile response of the urinary bladder in rats with streptozotocin-induced diabetes. *Jpn. J. Pharmacol.* **73**: 221–228.

Karahan, F., Alican, I., Ozkutlu, U., Onat, F., Yegen, B.C., Ulusoy, N.B. and Oktay, S. (1991). Muscarinic receptor subtypes of guinea-pig common bile duct. *Arch. Internat. Pharmacodyn. Ther.* **312**: 140–145.

Kortezova, N.,Shikova, L. and Papasova, M. (1998). Participation of M1 receptors in NO pathway in cat ileum. *Acta Physiol. Pharmacol. Bulgarica* **23**: 1–4.

Krichevsky, V.P., Pagala, M.K., Vaydovsky, I., Damer, V. and Wise, G.J. (1999). Function of M3 muscarinic receptors in the rat urinary bladder following partial outlet obstruction. *J. Urol.* **161**: 1644–1650.

Lambrecht, G., Gross, J. and Mutschler, E. (1999). Neuronal soma-dendritic and prejunctional M1-M4 receptors in gastrointestinal and genitourinary smooth muscle. *Life Sci.* **64**: 403–410.

Lin, S., Kajimura, M., Takeuchi, K., Kodaira, M., Hanai, H. and Kaneko, E. (1997). Expression of muscarinic receptor subtypes in rat gastric smooth muscle: effect of M3 selective antagonist on gastric motility and emptying. *Dig. Dis. & Sci.* **42**: 907–914.

Maeda, A., Tubo, T., Mishima, M. and Numa, S. (1988). Tissue distribution of mRNAs encoding muscarinic acetylcholine receptor subtypes. *FEBS Let.* **239**: 339–342.

Martin, S., Radley, S., Chess-Williams, R. and Chapple, C.R. (1997). Relaxant effects of potassium channel openers on normal and hyperreflexic human bladder. *BJU International.* **80**: 405–413.

Matsui, M., Motomura, D., Fujikawa, T., Jiang, J., Takahashi, S., Manabe, T. and Taketo, M.M. (2002). Mice lacking M2 and M3 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. and Taketo,

M.M. (2000). Multiple functional defects in peripheral autonomic organs in mice lacking muscarinic acetylcholine receptor gene for the M3 subtype. *Proc. Nat. Acad. Sci. U.S.A.* **97**: 9579–9584.

Michel, A.D. and Whiting, R.L. (1988). Methoctramine reveals heterogeneity of M2 muscarinic receptors in longitudinal ileal smooth muscle membranes. *Eur. J. Pharmacol.* **145**: 305–311.

Mimata, H., Nomura, Y., Emoto, A., Latifpour, J., Wheeler, M. and Weiss, R.M. (1997). Muscarinic receptor subtypes and receptor-coupled phosphatidylinositol hydrolysis in rat bladder smooth muscle. *Int. J. Urol.* **4**: 591–596.

Mimata, H., Wheeler, M.A., Fukumoto, Y., Takigawa, H., Nishimoto, T., Weiss, R.M. and Latifpour, J. (1995). Enhancement of muscarinic receptor-coupled phosphatidyl inositol hydrolysis in diabetic bladder. *Mol. Cel. Biochem.* **152**: 71–76.

Noronha-Blob, L., Lowe, V., Patton, A., Canning, B., Costello, D. and Kinnier, W.J. (1989). Muscarinic receptors: relationships among phosphoinositide breakdown, adenylate cyclase inhibition, in vitro detrusor muscle contractions and in vivo cystometrogram studies in guinea pig bladder. *J. Pharmacol. Exp. Therap.* **249**: 843–851.

Osinski, M.A. and Bass, P. (1994). Increased active stress generation of denervated rat intestinal smooth muscle: functional analysis of muscarinic receptor population. *J. Pharmacol. Exp. Ther.* **268**: 1368–1373.

Ostrom, R.S. and Ehlert, F.J. (1998). M2 muscarinic receptors inhibit forskolin- but not isoproterenol-mediated relaxation in bovine tracheal smooth muscle. *J. Pharmacol. Exp. Ther.* **286**: 234–242.

Peralta, E.G., Ashkenazi, A., Winslow, J.W., Ramachandran, J. and Capon, D.J. (1988). Differential regulation of PI hydrolysis and adenylyl cyclase by muscarinic receptor subtypes. *Nature* **334**: 434–437.

Pontari, M.A., Braverman, A.S. and Ruggieri, M.R. (2004). The M2 muscarinic receptor mediates in vitro bladder contractions from patients with neurogenic bladder dysfunction. *Am. J. Physiol.* **286**: R874–R880.

Preiksaitis, H.G. and Laurier, L.G. (1998). Pharmacological and molecular characterization of muscarinic receptors in cat esophageal smooth muscle. *J. Pharmacol. Exp. Ther.* **285**: 853–861.

Saito, M., Nakamura, I. and Miyagawa, I. (1997). Autoradiographic localization of muscarinic receptors in diabetic rat bladder. *Jpn. J. Urol.* **88**: 858–867.

Sellers, D.J., Yamanishi, T., Chapple, C.R., Couldwell, C., Yasuda, K. and Chess-Williams, R. (2000). M3 muscarinic receptors but not M2 mediate contraction of the porcine detrusor muscle in vitro. *J. Autonom. Pharmacol.* **20**: 171–176.

Shen, A. and Mitchelson, F. (1998). Muscarinic M2 receptor-mediated contraction in the guinea pig Taenia caeci: possible involvement of protein kinase C. *Bioch. Pharmacol.* **56**: 1529–1537.

Shi, X.Z. and Sarna, S.K. (1997). Inflammatory modulation of muscarinic receptor activation in canine ileal circular muscle cells. *Gastroenterol.* **112**: 864–874.

Sims, S.M., Clapp, L.H., Walsh, J.V. and Singer, J.J. (1990). Dual regulation of M current in gastric smooth muscle cells: beta-adrenergic-muscarinic antagonism. *Pflüg. Arch.–Eur. J. Physiol.* **417**: 291–302.

Somogi, G.T. and de Groat, W.C. (1999). Function, signal transduction mechanisms and plasticity of presynaptic muscarinic receptors in the urinary bladder. *Life Sci.* **64**: 411–418.

Somogi, G.T., Tanowitz, M. and de Groat, W.C. (1994). M1 muscarinic receptor-mediated facilitation of acetylcholine release in the rat urinary bladder. *J. Physiol. (Lond.)* **140**: 81–89.

Speakman, M.J., Brading, A.F., Gilpin, C.J., Dixon, J.S., Gilpin, S.A. and Gosling, J.A. (1987). Bladder outflow obstruction—a cause of denervation supersensitivity. *J. Urol.* **138**: 1461–1466.

Stengel, P.W., Gomeza, J., Wess, J. and Cohen, M.L. (2000). M2 and M4 receptor knockout mice: muscarinic receptor function in cardiac and smooth muscle in vitro. *J. Phamacol. Exp. Ther.* **292**: 877–885.

Stevens, L.A., Sellers, D.J., Chapple, C.R. and Chess-Williams, R. (2004). A comparison of muscarinic receptor function in the normal and neurogenic overactive bladder. *J. Urol. (Suppl)* **171**: 143, P535.

Togashi, H., Emala, C.W., Hall, I.P. and Hirshman, C.A. (1998). Carbachol induced actin reorganisation involves Gi activation of Rho in human airway smooth muscle. *Am. J. Physiol.* **274**: L803–L809.

Tong, Y.C., Chin, W.T. and Cheng, J.T. (1999). Alterations in urinary bladder M2-muscarinic receptor protein and mRNA in 2-week streptozotocin-induced diabetic rats. *Neurosc. Lett.* **277**: 173–176.

Wang, P., Luthin, G.R. and Ruggieri, M.R. (1995). Muscarinic acetylcholine receptor subtypes mediating urinary bladder contractility and coupling to GTP binding proteins. *J. Pharmacol. Exp. Therap.* **273**: 959–966.

Wang, Y.X., Fleischmann, B.K. and Kotlikoff, M.I. (1997a). M2 receptor activation of nonselective cation channels in smooth muscle cells: calcium and Gi/G(o) requirements. *Am. J. Physiol.* **273**: C500–C508.

Wang, Y.X. and Kotlikoff, M.I. (1997b). Muscarinic signaling pathway for calcium release and calcium-activated chloride current in smooth muscle. *Am. J. Physiol.* **273**: C509–C519.

Waring, J.V. and Wendt, I.R. (2000). Effects of streptozotocin-induced diabetes mellitus on intracellular calcium and contraction of longitudinal smooth muscle from rat urinary bladder. *J. Urol.* **163**: 323–330.

Weiser, M., Mutschler, E. and Lambrecht, G. (1997). Characterization of postjunctional muscarinic receptors mediating contraction in rat anococcygeus muscle. Naunyn-Schmeideb. *Arch. Pharmacol.* **356**: 671–677.

Wheeler, M.A., Martin, T.V. and Weiss, R.M. (1995). Effect of carbachol and norepinephrine on phosphatidyl inositol hydrolysis and cyclic AMP levels in guinea pig urinary tract. *J. Urol.* **153**: 2044–2049.

Yamaguchi, O., Shishido, K., Tamura, K., Ogawa, T., Fujimura, T. and Ohtsuka, M. (1996). Evaluation of mRNAs encoding muscarinic receptor subtypes in human detrusor muscle. *J. Urol.* **156**: 1208–1213.

Yamanishi, T., Chapple, C.R., Yasuda, K. and Chess-Williams, R. (2002). The role of M2-muscarinic receptor subtypes in mediating contraction of the pig bladder base following cyclic AMP elevation and/or selective M3-inactiviation. *J. Urol.* **167**: 397–401.

Yu, H.I., Wein, A.J. and Levin, R.M. (1997). Contractile responses and calcium mobilization induced by muscarinic agonists in the rat urinary bladder: effects of age. *Gen. Pharmacol.* **28**: 623–628.

Zhang, L.B. and Buxton, I.L. (1991). Muscarinic receptors in canine colonic circular smooth muscle. II. Signal transduction pathways. *Mol. Pharmacol.* **40**: 952–959.

(Received November 10, 2004; Accepted November 22, 2004)