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On the functional role of muscarinic M_2 receptors in cholinergic and purinergic responses in the rat urinary bladder

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Abstract

The functional effects of muscarinic receptor and purinoceptor agonists and antagonists were studied on isolated strip preparations of the rat urinary bladder. The muscarinic "M_3/M_1-selective" receptor antagonist 4-diphenylacetoxy-*N*-methylpiperidine methobromide (4-DAMP) most conspicuously inhibited the carbachol-evoked contractile responses ($pA_2 = 9.8$), while the muscarinic "M_1-selective" receptor antagonist pirenzepine and the muscarinic "M_2-selective" receptor antagonist methoctramine were less potent ($pA_2 = 7.0$ and 6.5, respectively). Administration of 4-DAMP in combination with methoctramine in selective dosages gave no significant additional reduction of carbachol-evoked contractile responses. Adenosine 5′-triphosphate (ATP) elicited transient dose-dependent contractile responses and it caused relaxation of the carbachol-contracted detrusor strips. The relaxatory response was enhanced in the presence of methoctramine and furthermore, was attenuated by the adenosine receptor antagonist 8-*p*-sulfophenyltheophylline. Administration of 2-chloro-adenosine to pre-contracted strips tended to cause dose-dependent relaxations, which were significantly increased in the presence of methoctramine. The purinergic contractile response, on the other hand, was not affected by methoctramine. Thus, the results are consistent with the cholinergic contractile response in the rat urinary bladder being exerted via activation of muscarinic M_3 receptors, while the muscarinic M_2 receptors exerted a modulator effect on purine-evoked relaxations in the rat urinary bladder.

Keywords: Muscarinic receptor subtype; Purinoceptor; Urinary bladder; (Rat)

1. Introduction

The parasympathetically nerve evoked contraction of the urinary bladder is mainly due to the action of acetylcholine via the activation of muscarinic M_3 receptors (see Andersson, 1993; Caulfield, 1993; Eglen et al., 1996). Conversely, in binding experiments, muscarinic M_2 receptors have been shown to be in vast majority on the detrusor (Lambrecht et al., 1989), but their functional role has just recently been partly unravelled. Thus, the activation of muscarinic M_2 receptors was shown to constrain β-adrenoceptor-mediated relaxation of rat urinary bladder (Hegde et al., 1997). Thereby, such an inhibitory effect on adrenergically mediated detrusor relaxation would facilitate voiding (Hegde and Eglen, 1999). An additional functional role of the muscarinic M_2 receptors of the bladder may be to elicit detrusor contraction by a direct mechanism, as suggested from in vivo studies on guinea-pig bladder (Sundquist, 1998). However, other neurotransmitters besides acetylcholine may contribute to detrusor excitation, since a substantial part of the parasympathetically evoked contraction in several species appears to be atropine resistant (Langley and Anderson, 1895; Burnstock et al., 1972; Husted et al., 1980; Sibley, 1984). Compelling evidence has been put forward showing the purine nucleotide, adenosine triphosphate (ATP) acting via P_2 purinoceptors, to be such a non-cholinergic neurotransmitter (Kasakov and Burnstock, 1982; Ziganshin et al., 1993; Ralevic and Burnstock, 1998). Adenosine, on the other hand, which is a purine nucleoside emerging from the metabolism of ATP, causes detrusor relaxation (Burnstock et al., 1972) via P_1 purinoceptors of the A_1 subtype (Stehle et al., 1992;

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Nicholls et al., 1992). Since several parasympathetic neurotransmitters/modulators may influence the micturition reflex (Andersson, 1993), the possible interactions of these substances via different receptors are numerous and may result in both contractile and relaxatory effects by a pre- as well as a postjunctional site of action (Somogyi and De Groat, 1992; Tobin and Sjögren, 1995; Somogyi et al., 1996; Tobin, 1998; Tobin and Sjögren, 1998).

The current study was designed to further investigate the functional roles of the bladder muscarinic M_2 receptors. The specific aims were to examine whether or not direct contractile effects via muscarinic M_2 receptors may occur in vitro, and furthermore, whether or not muscarinic M_2 receptors indirectly may cause contraction by modulating counteracting relaxatory stimuli other than those via the β-adrenoceptors. For this purpose, various muscarinic receptor antagonists were used (see Eglen and Watson, 1996): pirenzepine ("muscarinic M_1 receptor selective"; Hammer et al., 1980), methoctramine ("muscarinic M_2 receptor selective"; Melchiorre et al., 1987), 4-diphenylacetoxy-*N*-methylpiperidine (4-DAMP; "muscarinic M_3/M_1 receptor selective"; Barlow and Shepard, 1986; Doods et al., 1987;), and also the unselective adenosine receptor antagonist 8-*p*-sulfophenyltheophylline (Bruns et al., 1986).

2. Materials and methods

2.1. Surgical and experimental procedures

The Ethical Committee of Göteborg University approved the study design, in which 58 male rats (300–350 g) of the Sprague–Dawley strain were used. The rats were anaesthetized with pentobarbitone and the urinary bladder was removed. The rats were subsequently killed with an overdose of pentobarbitone. Full thickness strips (6×2 mm), with the average wet weight of 5.1 ± 0.2 mg ($n = 82$), were excised from the detrusor proximal to the orifices of the two ureters. The preparatory methods were generally as described previously (Tobin and Sjögren, 1995). For the contraction experiments, the detrusor strip was mounted between two steel rods, of which one was fixed and the other adjustable. The strips were immersed in 20-ml organ baths containing Krebs bicarbonate solution (pH = 7.25) of the following composition (mM): NaCl 118, KCl 4.6, $CaCl_2$ 1.25, KH_2PO_4 1.15, $MgSO_4$ 1.15, $NaHCO_3$ 25, and glucose 5.5, which was gassed with 5% CO_2 in O_2 (a high K^+ solution containing 124 mM K^+ was obtained by exchanging Na^+ for equimolar amounts of K^+). The temperature was kept at 37 °C by a thermostat. The detrusor preparations were pre-stretched, which resulted in gradual tension relaxation. The preparations were repeatedly stretched so that a stable tension of about 3 mN was obtained after 30–45 min. A reference carbachol concentration (10^{-5} M) was initially administered at least twice in each experiment, before and after the renewal of the Krebs buffer. In cases of the discrepancy between the responses exceeding 5%, the administration was repeated until a stable response was achieved (interval of 5 min). The contractile responses were expressed in percentage of the last reference response obtained.

All drugs were administered to the organ baths in a volume of 100 μl. The agonists administered were carbachol and/or ATP or 2-chloro-adenosine, and the antagonists used were pirenzepine, methoctramine, 4-DAMP and/or 8-sulfophenyltheophylline. The antagonists were administered 20 min prior to addition of the agonists. When the effect of ATP was administered in combination with carbachol, the nucleotide was added to the bath just prior to the administration of carbachol (see Fig. 1). In the testing of 2-chloro-adenosine, the administration of the nucleoside was performed when a relatively stable plateau of the response to the single concentration of carbachol was reached, which occurred within 2 min after the peak of the response. The peak of the response to carbachol was reached within 30 s upon carbachol administration, and after two min tension had declined to a level of 85–75% of the peak response. The concentration of carbachol was 10^{-6} M when a single carbachol concentration was used, since this concentration gives a response at the log phase in the concentration–response curve.

2.2. Materials

The drugs (purchased from Sigma, St. Louis, US, if not otherwise stated) administered during the experiments were as follows: adenosine 5′-triphosphate (ATP), carbamylcholine chloride (carbachol), 2-chloro-adenosine, 4-diphenylacetoxy-*N*-methylpiperidine methobromide (4-DAMP; a kind gift from Dr Barlow, University of Bristol, UK), methoctramine hydrochloride Research Biochemicals, Natick, US), pirenzepine dihydrochloride, 8-*p*-sulfophenyltheophylline. All concentrations mentioned in the text refer to the final ones in the organ bath chambers.

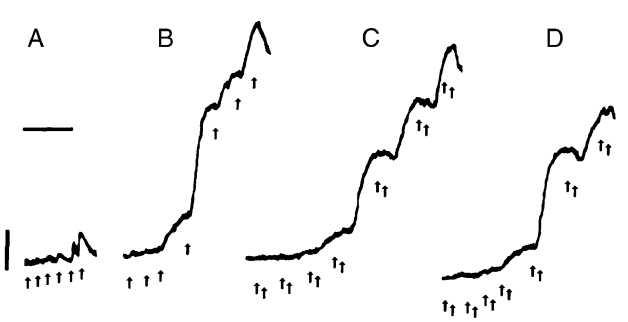

Fig. 1. Recordings of contractile responses from a single preparation to ATP alone ((A) 5×10^{-6} to 5×10^{-3} M), and to carbachol alone ((B) 5×10^{-8} to 5×10^{-5} M), and to carbachol after ATP (10^{-5} M; first arrow in each pair), before (C) and after (D) administration of methoctramine (5×10^{-8} M). Vertical bar shows the time period of 5 min and the horizontal bar a change of tension by 5 mN.

2.3. Calculations and statistics

Schild plots for competitive antagonism were constructed from the dose ratios (DR) of full carbachol response curves, obtained from at least three different antagonist concentrations [B], to estimate the p$A2$ values (Arunlakshana and Schild, 1959). For each antagonist and set of conditions, log(DR-1) was plotted against log[B]. Statistical significance was determined by Student's t-test for unpaired data or for paired data when relevant. When multiple comparisons with the same variable were made, a t-test according to the Bonferroni method was used (Wallenstein et al., 1980). P values of 0.05 or less were regarded as statistically significant. Data are presented in the form of means ± S.E.M., and n refers to number of preparations. Graphs were generated and parameters computed using the Microsoft® Excel program.

3. Results

3.1. Carbachol-evoked responses

Carbachol elicited almost instant contractile responses of the urinary bladder strip preparations when administered above a threshold concentration of 5×10^{-8} to 5×10^{-7} M. The maximal contractile response of the isolated muscle strips was 4.6 ± 0.2 mN/mg tissue wet weight ($n = 82$) and was elicited at a concentration of 5×10^{-6} to 10^{-5} M. The EC_{50} value for carbachol-evoked contractions was 2.28 ± 0.21 μM, and 4-DAMP, methoctramine and pirenzepine all gave rise to right shifts of the carbachol-evoked concentration–response curves (Fig. 2A–D). 4-DAMP most conspicuously shifted the curve to the right and Schild analysis generated the pA_2 value for 4-DAMP to 9.8 ± 0.4 ($n = 6$), while the pA_2 values for the less potent

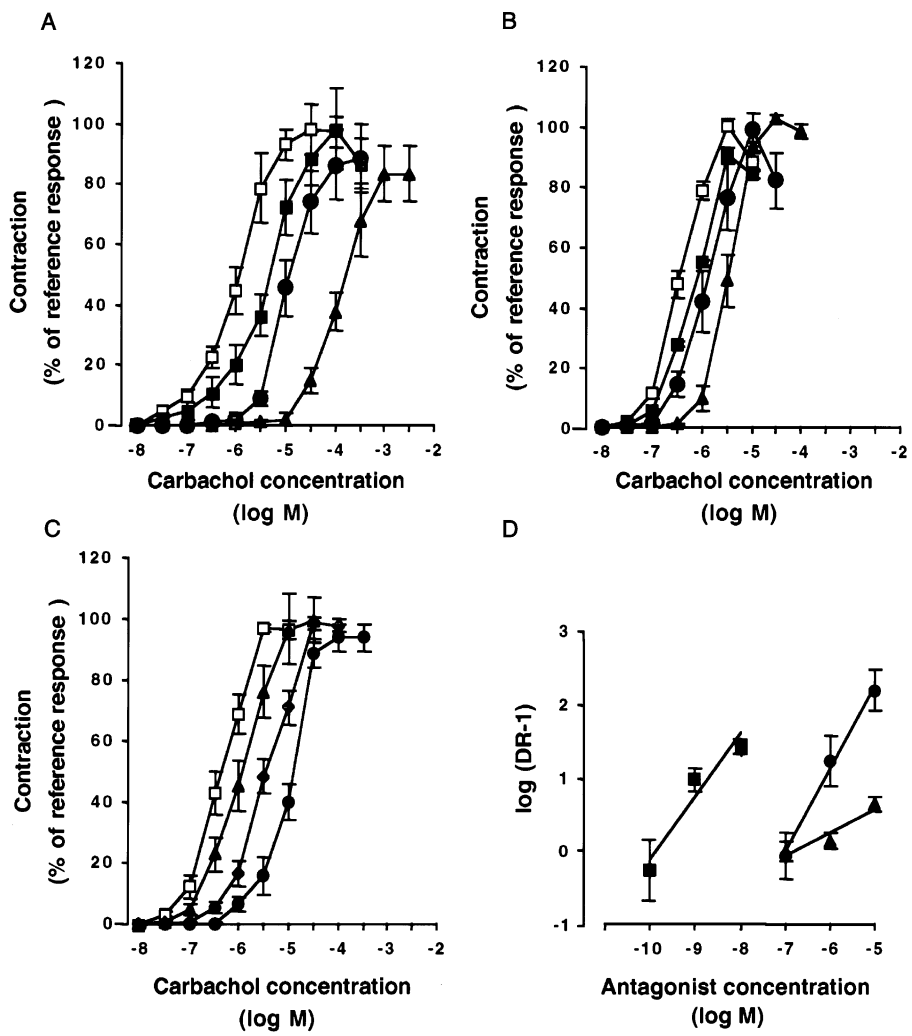

Fig. 2. Panels (A)–(C). Mean contractile responses to carbachol of isolated detrusor strips in the absence of antagonists (□) and in the presence of (A) pirenzepine ($n = 6$; ■ 10^{-7} M; ● 10^{-6} M; ▲ 10^{-5}M); (B) methoctramine ($n = 5$; ■ 10^{-7} M; ● 10^{-6} M; ▲ 10^{-5} M); and (C) 4-DAMP ($n = 6$; ▲ 10^{-10} M; ♦ 10^{-9} M; ● 10^{-8} M). Contractile responses are expressed as percentages of an initially evoked reference carbachol response (10^{-5} M). Panel (D): Schild regressions for the antagonism in panels (A)–(C) (●: pirenzepine, 1.1 ± 0.1 (slope ± S.E.M.); ▲: methoctramine, 0.4 ± 0.1; ■: 4-DAMP, 0.9 ± 0.1). Vertical bars represent S.E.M.

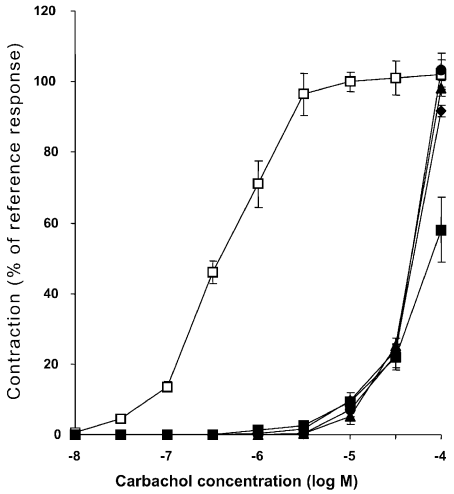

Fig. 3. Mean contractile responses to carbachol of isolated detrusor strips in the absence of antagonists (□) and in the presence of methoctramine (● 10^{-8} M; ▲ 5×10^{-8} M; ♦ 10^{-7} M; ■10^{-6} M) and 4-DAMP (10^{-8} M; $n=4$) at different concentrations. Contractile responses are expressed as percentages of an initially evoked reference carbachol response (10^{-5} M). Vertical bars represent S.E.M.

antagonists pirenzepine and methoctramine were 7.0 ± 0.3 ($n=6$) and 6.5 ± 0.3 ($n=6$), respectively. The slopes of the Schild plots were not significantly discrepant from unity for 4-DAMP and pirenzepine, whereas that for methoctramine differed ($P<0.05$). Administration of 4-DAMP (10^{-9} and 10^{-8} M) in combination with low concentrations of methoctramine (5×10^{-9}, 10^{-8}, 5×10^{-8}, 10^{-7} M) gave no additional inhibition of the contractile carbachol-evoked contractions (Fig. 3; shows inhibitory effects of methoctramine in combination with 4-DAMP at 10^{-8} M). However, when 4-DAMP (10^{-8} M) was given in combination with methoctramine at 10^{-6} M (cf. $pA_2 = 6.5$) the contractions were additionally inhibited ($-42 \pm 9\%$ vs. $-4 \pm 4\%$ by 4-DAMP alone, at carbachol 10^{-4} M).

3.2. ATP-evoked responses

ATP elicited transient concentration-dependent contractile responses, with a threshold concentration of 10^{-7} to 10^{-6} M, while maximal effect was noted at 5×10^{-3} M (0.3 ± 0.05 mN/mg, $n=8$). Administration of a relatively low ATP concentration (10^{-5} M) in combination with carbachol tended to shift the carbachol-evoked concentration–response curve to the right. Significantly smaller carbachol-evoked contractile responses emerged from the challenge with ATP in combination with carbachol concentrations higher than 10^{-6} M (Figs. 1 and 4; $P<0.05$–0.01), suggesting that ATP also evokes relaxations. If so, such may result from the action of adenosine produced by the breakdown of ATP (Schaufele et al., 1995). This hypothesis was tested by pre-incubating the preparations with the adenosine receptor antagonist 8-*p*-sulfophenyltheophylline (10^{-6} M). In the presence of this agent, the seemingly relaxatory effect of ATP on the carbachol-evoked responses tended to be attenuated. At 10^{-5} M of carbachol, the ATP evoked reduction of the contractile response was inhibited by -72% ($P<0.05$; $n=10$). Methoctramine (5×10^{-8} M), on the other hand, quantitatively increased the relaxatory response to ATP (Fig. 4). Whereas the relaxatory effect by ATP on the contractile response to carbachol at 10^{-6} M was $-58 \pm 3\%$ ($n=18$) in the absence of methoctramine, the corresponding reduction in its presence (5×10^{-8} M) was $-87 \pm 4\%$ ($P<0.001$). The antagonist 8-*p*-sulfophenyltheophylline (10^{-6} M) also conspicuously attenuated the ATP-evoked relaxations in the presence of methoctramine (5×10^{-8} M); the reduction was inhibited by 73% at 10^{-5} M of carbachol ($P<0.05$; $n=10$). The contractile part of the ATP-evoked response was not affected by methoctramine. ATP (10^{-5} M) also caused relaxation by $20 \pm 5\%$ ($n=10$) of strip preparations pre-contracted by K^+ Krebs solution (7 ± 1 mN at 50 mM). This relaxation was not affected by methoctramine.

3.3. Adenosine-evoked responses

The agonist 2-chloro-adenosine, when administered non-cumulatively at the concentrations of 10^{-8}, 5×10^{-8} and 10^{-7} M to pre-contracted strips (carbachol 10^{-6} M), tended to cause concentration-dependent relaxations (up to -30% from peak; $n=5$). However, no statistical significance was attained when these relaxations were compared with the decline of the carbachol-induced tension in the absence of 2-chloro-adenosine ($-18.7 \pm 5.5\%$ 2 min after

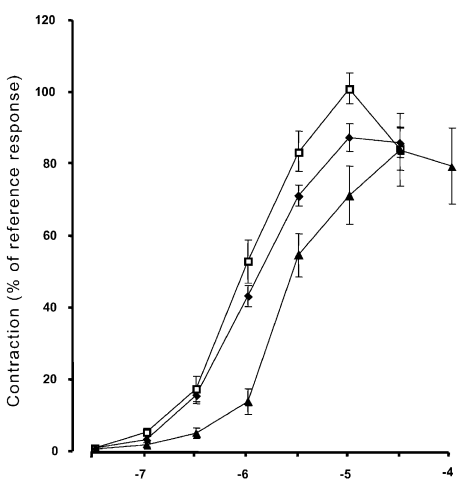

Fig. 4. Mean contractile responses ($n=18$) to carbachol alone (□), to carbachol after administration of ATP (10^{-5} M; ♦) and to carbachol after administration of ATP (10^{-5} M) in the presence of methoctramine (5×10^{-8} M; ▲) of isolated detrusor strips at different carbachol concentrations. Contractile responses are expressed as percentages of an initially evoked reference carbachol response (10^{-5} M). Vertical bars represent S.E.M.

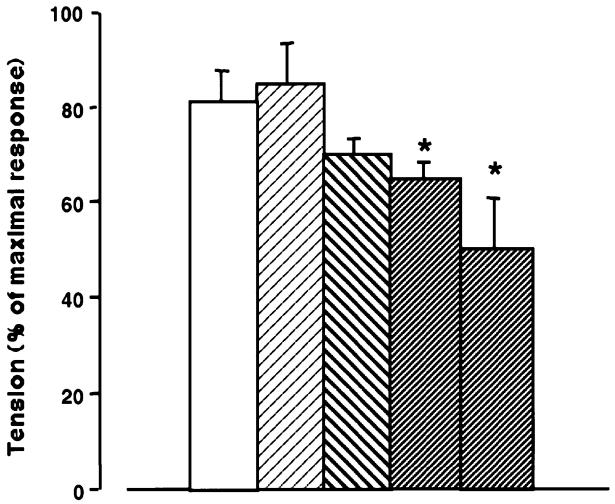

Fig. 5. Mean contractile tensions of isolated detrusor strips 2 min after the peak of the response to carbachol (5×10^{-6} M) in the absence (□) and presence of methoctramine (▨; 10^{-7} M; $n=4$), 2-chloro-adenosine (▧; 5×10^{-8} M; $n=6$), and 2-chloro-adenosine + methoctramine (▨; 5×10^{-8} M (▨; first column from the left) and 10^{-7} M (▨; second column), respectively; $n=6$). Tensions are expressed as percentages of the peak contraction. Vertical bars represent S.E.M.; $^{*}P<0.05$, in comparison with the decline in the absence of 2-chloro-adenosine and methoctramine.

peak). For a more profound examination of the 2-chloro-adenosine effects in the absence and presence of methoctramine, the concentration of 5×10^{-8} M of 2-chloro-adenosine was selected. The preparations were challenge by this concentration of 2-chloro-adenosine initially as well as at the end of the testing protocol, whereupon the administrations resulted in the same tension reductions ($-28.4\pm3.4\%$ vs. $-27.5\pm3.1\%$; first and last administrations, respectively; $n=6$). In the presence of methoctramine at 5×10^{-8} and 10^{-7} M ($n=6$ at each concentration), on the other hand, the 2-chloro-adenosine-evoked (5×10^{-8} M) relaxations were substantially and significantly enhanced ($-36.0\pm3.0\%$ and $-47.8\pm9.8\%$, respectively; $P<0.05$; Fig. 5). Methoctramine at lower concentrations (5×10^{-9}, 10^{-8} M; $n=6$) did not affect the 2-chloro-adenosine-evoked relaxations. Furthermore, in control experiments on carbachol-contracted preparations, conducted in the absence of 2-chloro-adenosine but in the presence of methoctramine 10^{-7} M, the decline in tension did not differ from that of preparations with only carbachol present.

4. Discussion

The present study indicates that the cholinergic contractile response in the rat urinary bladder is exerted via the activation of a homogenous population of muscarinic receptors, which belong to the muscarinic M_3 receptor subtype. We found no evidence for a direct muscarinic M_2 receptor mediation of cholinergic contractions. However, a modulating, inhibitory, role for the numerously occurring muscarinic M_2 receptors of purinoceptor-mediated relaxation of the detrusor was demonstrated. In contrast, the muscarinic M_2 receptors did not affect purinergic contractile responses. Furthermore, the relaxatory part of the ATP induced biphasic response seemed, at least to some part, to be due to effects of products from the breakdown of ATP acting on P_1 purinoceptors.

The inhibitory potencies of the muscarinic antagonists on carbachol-evoked contractile responses of the urinary bladder strips showed resemblance with the potencies reported previously (Longhurst et al., 1995; Wang et al., 1995; Hegde et al., 1996; Tobin, 1995; Tobin and Sjögren, 1995). The Schild analysis revealed 4-DAMP to inhibit carbachol-evoked contractions 2000 times more potently than methoctramine and 600 times more potently than pirenzepine. The differences between the pA_2 values for the antagonists are consistent with a tissue expressing only functional muscarinic M_3 receptors, and further and notably, neither of the slopes of the Schild plots for 4-DAMP nor pirenzepine differed from unity. The slope of the Schild plot for methoctramine, on the other hand, differed from unity, but this difference seems most likely to be referred to qualities of the antagonist and not to the receptor population. Contextually noteworthy, competitive antagonists have been reported to behave non-competitively at high concentrations (Melchiorre, 1988).

Studies on contractile mechanisms in the gastrointestinal tract of the guinea pig have shown a direct contractile effect of the muscarinic M_2 receptor subtype during blockade of the muscarinic M_3 receptor (Ehlert et al., 1999). Therefore, it was presently wondered whether the contractile effect of carbachol, when administered during concomitant muscarinic M_3 receptor blockade (i.e., in the presence of different concentrations of 4-DAMP), would be further attenuated by muscarinic M_2 receptor antagonism (i.e., methoctramine). For this purpose, methoctramine was administered at concentrations lower than 10^{-6} M, at which, as the present data indicated, a selective blockade of the muscarinic M_2 receptors occurs, and those concentrations did not result in further inhibition. At larger concentrations of methoctramine the inhibitory effect of 4-DAMP was enhanced, but a non-selective receptor blockade may thus have occurred. All in all, this may be interpreted as contractions evoked by cholinergic stimulation are exerted via activation of a homogenous muscarinic receptor population. If a direct muscarinic M_2 receptor contractile response still occurs, this is of such a minute magnitude that it would not have affected the interpretation of the muscarinic M_2 receptor modulation of the purinergic responses. Also, in mice lacking the muscarinic acetylcholine receptor gene for the muscarinic M_3 receptor subtype, a direct contractile response to carbachol was suggested, but, similarly to the present results, the authors were not able to pharmacologically characterize the response (Matsui et al., 2000).

Conversely, in a number of studies, including the methodology of multivariate analysis for characterization of muscarinic receptor subtypes, Sundquist (1998) demonstrated a contractile effect of the muscarinic M_2 receptor subtype, in vivo, in guinea-pig urinary bladder. Notably, this author emphasized that the contractile capacity of the muscarinic M_2 receptor was compellingly smaller in vitro, than in vivo. In the view of carbachol being a nicotinic, as well as muscarinic receptor agonist (Boksa et al., 1989), this compound may have activated postganglionic nerve fibres, in vivo, which, at least in part, could account for the difference observed. That finding made us address the problem whether the muscarinic M_2 receptors on the detrusor may have a similar opposing effect on parasympathetically evoked relaxatory responses, as on β-adrenoceptor-mediated relaxations (Hegde et al., 1997; Hegde and Eglen, 1999). In this context, ATP seems to be of particular interest, since this purine nucleotide was demonstrated to co-exist with acetylcholine in the parasympathetic neurons of the urinary bladder (Ziganshin et al., 1993; Theobald, 1996), and furthermore, since the metabolism of ATP may produce adenosine (Schaufele et al., 1995). Consequently, indirect relaxatory effects may be induced by ATP (Burnstock et al., 1972).

ATP, when administered to the isolated urinary bladder of several species including rat, evokes a transient contraction followed by a sustained relaxation (Bolego et al., 1995). Also in the present study, ATP elicited a dual response, of which the relaxatory component was most evident when ATP was administered to preparations having been pre-contracted by carbachol. The adenosine receptor antagonist 8-*p*-sulfophenyltheophylline largely attenuated the ATP-evoked relaxation. This relaxation, therefore, seems at least partly to be due to the breakdown of ATP to adenosine, exerting smooth muscle relaxation via P_1 purinoceptors. Our finding may not be in conflict with that of Bolego et al. (1995), who reported that 8-(*p*-sulfophenyl)-theophylline did not affect the ATP relaxation, since these authors did not investigate the pre-contracted preparations. The reasons for the disparate findings in the studies doubtless hinge on the differences in methodology. Currently, the effect of ATP was studied on strips contracted by carbachol, while Bolego et al. (1995) studied the effect on the basal tension of the bladder strips. Interestingly, Suzuki and Kokubun (1994) also found 8-*p*-sulfophenyltheophylline to inhibit the ATP evoked relaxation of rat detrusor strips contracted by acetylcholine, whereas the antagonist was without effect when studied on marmoset detrusor strips at basal tension (McMurray et al., 1998). Seemingly, a pre-requisite for the theophylline-blocking effect of ATP-induced detrusor relaxation to be apparent is that pre-contracted preparations are investigated. We may, moreover, conclude that a certain production of adenosine by ATP degradation is likely to occur in the bladder, and which may cause relaxation.

Currently, the effects of muscarinic M_2 receptors on purinergic responses, and in particular the relaxations, of the detrusor were thus addressed in the perspective of muscarinic M_2 receptor antagonism possibly interfering with parasympathetic contractile responses. An inhibitory effect on direct or indirect relaxatory effects by other parasympathetic transmitters than acetylcholine could clearly be one explanation for an antagonistic effect of muscarinic M_2 receptor activation being evident in vivo, but hardly in vitro. Therefore, in the examination of possible muscarinic effects on purinergic relaxatory responses, 2-chloro-adenosine was administered to strips pre-contracted by carbachol. Although no significant responses were observed, tendencies to dose-dependent relaxatory responses occurred, but most importantly, administration of 2-chloro-adenosine in the presence of methoctramine-induced substantial and significant relaxations of the carbachol-contracted strips. In this context the more pronounced ATP-induced relaxations in the presence of methoctramine are noteworthy and consistent with our proposal that ATP generated products which, in turn, activate adenosine receptors (i.e., P_1-purinoceptors). Although methoctramine being present at a concentration that seemingly exerted selective muscarinic M_2 receptor blockade, a certain direct muscarinic M_3 receptor antagonism could of course also have contributed to the reduction of the tension. Nevertheless, the muscarinic M_2 receptor exerts an inhibitory effect on the 2-chloro-adenosine-evoked relaxatory effects, which has semblance of that on β-adrenoceptor evoked relaxation of the detrusor. With respect to the purine relaxation, it may tentatively be suggested that the muscarinic M_2 receptor interfere intracellularly by inhibiting adenosine A_{2B} receptor-induced production of second messenger (cAMP), which normally leads to relaxation of the detrusor cells. Such an effect of muscarinic M_2 receptors on adenylate cyclase is well-documented biochemically (Caulfield, 1993). Endogenous transmitter acting on modulator muscarinic M_2 receptors, simultaneously to exerting contractile effects via muscarinic M_3 receptors, activate most likely a mechanism by which the detrusor cell eliminates a counteracting relaxatory effect via P_1 purinoceptors. Notably, methoctramine did not affect ATP evoked relaxations of potassium pre-contracted strip preparations, indicating that muscarinic effects are pre-requisites for the methoctramine-induced enhancement of ATP relaxation.

Thus, the present experiments demonstrate effects via the muscarinic M_2 receptors, i.e. a modulatory role for the muscarinic M_2 receptors on effects elicited via ligation of adenosine receptors that may be interpreted as an antagonism of contractions caused by stimulation of parasympathetic nerves, in vivo. Furthermore, other mechanisms, such as the occurrence of (prejunctional) facilitatory and inhibitory receptors on parasympathetic nerve terminals (Somogyi and De Groat, 1992; Somogyi et al., 1996; Tobin and Sjögren, 1998; Tobin, 1998) may also complicate the interpretation of results from in vivo investigations.

The presently suggested mechanisms, which are activated by muscarinic M_2 receptors in the rat urinary bladder and which seem to be of at least similar significance as the suggested direct contractile effects, are favoured by the following principal findings. First, the adenosine-evoked relaxation of carbachol-precontracted strips was significantly enhanced by muscarinic M_2 receptor blockade. Second, this muscarinic M_2 receptor blockade had no effect in the absence of purine agonists, and third, the muscarinic M_2 receptor blockade also enhanced ATP-induced relaxation. However, unraveled factors may of course play an unpredictable role, such as a direct muscarinic M_2 receptor contractile effect. Presently, the methoctramine inhibition of carbachol-evoked contraction was interpreted as unselective blockade occurring at large doses of the antagonist. A direct effect, if existing, seems as mentioned above, to be of a minute magnitude.

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