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Muscarinic receptors mediating contraction of female mouse urinary bladder: effects of oestrogen

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Abstract

Muscarinic receptors mediating contraction of bladder detrusor muscle from female mice were examined. Mice were untreated (A) or treated with oestradiol cypionate (200 μg/kg) 24 h (B) or 96 h (C) before experimentation, or were pregnant (day 17) (D). Saturation radioligand binding experiments using [^{3}H]quinuclidinyl benzilate ([^{3}H] QNB) indicated similar muscarinic receptor densities and affinities in bladders from groups A and B. Neither oestrogen treatment nor pregnancy altered pD_2 estimates for methacholine. Maximum responses to methacholine and high-K^+ physiological salt solution (KPSS) were significantly greater ($P<0.05$) in tissues from groups C and D than in A and B. Potencies of other muscarinic receptor agonists were similar in groups A and B with an order of acetylcholine plus physostigmine (10 μM) ≈ methacholine plus physostigmine (10 μM)>methacholine ≈ acetylcholine>bethanechol. Antagonist pK_B estimates were similar in bladders from groups A and B with a rank order of: atropine ≥ 4-diphenyl acetoxy-*N*-methyl piperidine methiodide>parafluorohexahydrosiladifenidol ≈ pirenzepine>himbacine, implicating muscarinic M_1 and/or M_5 as well as muscarinic M_3 receptors in mediating methacholine-induced bladder contraction.

Keywords: Bladder; Female mouse; Oestrogen; Muscarinic M_1, M_3 and M_5 receptors; Pregnancy

1. Introduction

The ability of the bladder detrusor muscle to produce a sustained contraction is essential for bladder emptying. The release from parasympathetic fibres of acetylcholine that then interacts with muscarinic receptors on the detrusor muscle surface is predominantly responsible for sustained contraction. Concomitant ATP release produces, in the rodent bladder, a more transient effect (Burnstock et al., 1972, 1978).

Urinary incontinence is a common condition in women, affecting 10–25% of adult women. Lack of bladder control is often due to dysfunction of the detrusor, the urethral sphincter mechanism, or both (Fantl et al., 1996). Multiple aetiological factors may lead to such disorders, and there is considerable evidence to support the hypothesis that hypoestrogenism can be a factor in development of dysfunctions of the urethra and bladder (Fantl et al., 1996). In the present investigation, we have examined whether oestrogen treatment modifies muscarinic receptor mediated effects in the bladder of the female mouse. We have chosen this species as it affords the possibility of using genetically modified animals such as gene knockouts for studying dysfunctions of the bladder (Bassuk et al., 2000; Bymaster et al., 2001; Matsui et al., 2000, 2002). In the male mouse detrusor muscarinic M_3 receptors are the major, but not the exclusive, subtype mediating contractile responses to muscarinic receptor agonists (Choppin and Eglen, 2001). In the female there are strain differences (Choppin, 2002) but a preliminary study from this laboratory (Paravicini et al., 2000) as well as the later study by Choppin (2002) indicated that a muscarinic M_3 receptor exclusively mediated methacholine-induced contractions in female mouse bladder.

The principal aims of this experiment were (a) to characterise the receptors mediating the contractile effects of muscarinic receptor agonists on the female mouse bladder and (b) to examine the effects of short-term exposure to oestrogen on muscarinic receptor agonist and antagonist

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potencies, and on muscarinic receptor density. A limited subset of experiments using bladder from 17-day pregnant mice was also undertaken.

A preliminary account of this data has been communicated previously (Ma et al., 2000).

2. Materials and methods

2.1. Animals and treatments

The Monash University Animal Ethics Committee approved the procedures outlined.

Virgin female Balb-C mice were housed at 22 °C with a continuous photoperiod of 12-h light and 12-h dark with free access to standard rodent food pellets and water ad libitum. The mice used were assigned to up to four experimental groups: (A) untreated mice (15–20 g); (B) 1-day oestrogen-treated mice (15–20 g) treated 24 h prior to experimentation with a single 0.06 ml subcutaneous injection of oestradiol cypionate in peanut oil 200 μg/kg; (C) 4-day oestrogen-treated mice (15–20 g) treated 96 h prior to experimentation with a single 0.06 ml subcutaneous injection of oestradiol cypionate in peanut oil 200 μg/kg; and (D) late pregnant; pregnant Balb-C mice (≈30 g) were used on day 17 of the 18- to 20-day gestation period.

Animals were killed by inhalation of carbon dioxide, followed by exsanguination, after which the abdomen was opened and the bladder body removed carefully and immediately placed in modified Krebs–Henseleit solution comprising (mmol/l): NaCl (118.0); KCl (4.7); $MgSO_4$ (1.1); KH_2PO_4 (1.18); $NaHCO_3$ (25.0); glucose (11.66); and $CaCl_2.2H_2O$ (1.9) on ice.

Vaginal smears were taken to confirm cornification of the vaginal epithelium in the oestrogen-treated group and to determine cycle stage in the untreated group. Smears were air-dried and fixed with methanol prior to staining overnight with Giemsa stain and then rinsed in cold water.

2.2. Saturation radioligand binding studies

The general protocol for this series of experiments was based on that described previously (Pennefather et al., 1994). The bladders were obtained as described above, and were dissected out and placed immediately in ice-cold Na_2HPO_4 sodium phosphate buffer (50 mM, pH 7.4 adjusted with 1.0 M HCl).

Four mice, either untreated or oestrogen-treated, provided sufficient homogenate for each binding experiment. The tissues were dissected free of connective tissue and fat, blotted dry on paper towel and collectively weighed. Ice-cold sodium phosphate buffer was used for all drug dilutions, homogenisation and for washing filters. Bladders were homogenised using the Ultra-Turrax Homogeniser at setting 185 with two homogenisations of 30 s bursts at 2-min intervals, resting on ice in 11 ml buffer. The resulting homogenate was centrifuged using a Sorvall SS-34 rotor centrifuge at 3000 rpm for 12 min at 4 °C. The supernatant was then carefully pipetted out. Samples were assayed in triplicate. Each 1 ml assay tube contained 150 μl membrane homogenate, and 800 μl buffer (± atropine 10 μM). Total binding was determined in the absence of atropine. Non-specific binding was determined by incubating the membrane fraction and radioligand with atropine (10 μM). All tubes were preincubated for 10 min in a shaking water bath at 37 °C. Radioligand ([^{3}H]QNB 20–400 pM, specific activity 48.0 Ci/mmol) was then added to each sample (50 μl) and further incubated at 37 °C for 60 min.

A Whatman glass microfibre filter (GF/B) presoaked overnight in 0.1% polyethyleneimine and atropine (10 μM) was fitted in a Brandel cell harvester and each assay tube was filtered with approximately 5 ml ice-cold buffer solution twice. The bound ligand trapped on the filter was placed in tubes with a suspension of Ecolite scintillant (4 ml) overnight before being counted in a United Technologies Packard Tri-Carb 4000-series liquid scintillation counter. Disintegrations per minute (DPM) were counted over 5 min for each tube. Total counts were determined from vials containing scintillant and radioligand only.

2.3. Functional studies

As described by Durant et al. (1991), whole bladder preparations were used for organ bath studies and each constituted a single experiment since one animal provided one preparation. The tissues were mounted vertically in separate 10 ml organ baths containing modified Krebs–Henseleit solution. A MacLab data acquisition system was used for measuring and recording force. Baths were maintained at a constant 37 °C temperature and continuously bubbled with carbogen (95% O_2, 5% CO_2). Resting tension was found to be optimal at 2 g and the tissues were allowed an initial equilibration period of at least 20 min.

Log concentration–response curves to agonist drugs in the absence and presence of antagonist drugs were constructed by the cumulative method. Agonist concentrations ranged from 10 nM to 300 μM. Tissues were subjected to three log concentration–response curves using a single agonist, with 1 h elapsing between successive curves; during this time the tissues were washed by overflow every 15 min. In experiments to determine effects of antagonists, one concentration–response curve was constructed in the absence of antagonist, then preparations were exposed to the lower of two concentration of antagonist during the 1 h incubation period and the log concentration curve repeated. The higher concentration of antagonist was applied for the 1 h incubation period and a third curve agonist curve constructed. Otherwise treatment was consistent with the time controls with washing and replacing antagonist or vehicle every 15 min. At the end of the experiments tissues were exposed to a high-K^+ phys-

iological salt solution (KPSS) in which the K^+ concentration was increased to 40 mM and the Na^+ concentration decreased to 70 mM. Tissues were weighed on completion of experiments.

2.4. Drugs

Acetyl-β-methylcholine chloride (methacholine chloride), Sigma; acetylcholine chloride, Sigma; carbamyl-β-methacholine chloride (bethanechol), Sigma; atropine sulfate, Sigma; 4-diphenyl acetoxy-*N*-methyl piperidine methiodide (4-DAMP), RBI; pirenzepine dihydrochloride, Boehringer Ingelheim; himbacine hydrochloride, a gift from Prof. Taylor, University of Sydney; *para*-fluorohexahydrosiladifenidol (*p*-F-HHSiD), RBI; 1-quinuclidinyl [phenyl-4-^{3}H] benzilate ([^{3}H]QNB) specific activity 48.0 Ci/mmol, Amersham; oestradiol cypionate-17-β, Sigma; polyethyleneimine, Sigma; physostigmine (eserine), Sigma. All agonists and antagonists except *p*-F-HHSiD and physostigmine were diluted in distilled water to produce 10 mM stock solutions that were stored at 0 °C. *p*-F-HHSiD was dissolved in absolute ethanol. Physostigmine was dissolved in catecholamine diluent comprising (mmol/l): NaCl (154); NaH_2PO_4 (1.86); and ascorbic acid (0.22). Oestradiol cypionate-17-β was first dissolved in 0.1 ml ethanol and further diluted in 10 ml peanut oil and stored wrapped in aluminium foil due to light sensitivity. Concentrated [^{3}H]QNB was kept in an opaque plastic storage container in the freezer and was diluted in sodium phosphate buffer when needed. Atropine sulphate used in radioligand binding experiments was dissolved in sodium phosphate buffer. Polyethyleneimine was dissolved thoroughly in distilled water before soaking glass microfibre filters.

2.5. Data analysis and statistical treatment

Unless otherwise specified, *n* refers to the number of animals used. In all comparisons, a *P* value <0.05 was accepted as an index of statistical significance.

Saturation binding data were analysed using GraphPad Prism v3.0, using a saturation binding template. Data were standardised for time-dependent changes in specific activity of the radioligand and differences in total tissue weight. Final specific activity was determined using RADLIG (McPherson, 1983), then raw saturation binding counts (disintegrations per min, (DPM)) were standardised to fmol [^{3}H]QNB/mg tissue weight. Mean B_{max} and K_d were calculated using GraphPad Prism, and a Student's unpaired two-tailed *t*-test was performed to compare corresponding values between two treatment groups. GraphPad Prism v3.0 also enabled statistical comparisons to be made between one- and two-site fit models.

Functional data were evaluated by the computer program GraphPad Prism v3.0 and estimates were expressed as mean ± standard error of the mean (S.E.M.). Tissue variability was corrected for by expressing all concentration–response curves as a percentage of the maximum value obtained during the first control curve, since an increase in bladder contractile response with repetition of concentration–response curves was generally observed. GraphPad Prism v3.0 was used to estimate nH to determine whether slopes in the presence of antagonists or vehicle were parallel to their respective controls, and agonist pD_2 values (negative logarithm of the molar concentration of agonist producing 50% of the maximum response) were estimated. When log concentration curves did not asymptote over the range of concentrations added, pEC_{50} values were determined where EC_{50} equals the concentration producing 50% of the maximum response observed. Mean values for agonists in the absence and presence of antagonists were calculated. Concentration ratios (CRs) were determined from pD_2 estimates in the absence and presence of antagonists. pK_b values were calculated as (pK_b = negative logarithm [antagonist]/$CR-1$) (Furchgott, 1972). One-way analyses of variance were conducted using GraphPad Instat 3.01 for group comparisons, followed by Tukey–Kramer comparisons of mean values. One-way repeated measures analysis followed by Tukey–Kramer comparisons of mean pK_b values was used to determine antagonist-induced shifts in log concentration–responses curves. Comparisons between mean estimates in control and 24 h oestrogen primed groups were made using Student's unpaired two-tailed *t*-test. Pearson correlation coefficients (*r*) and associated *P* values were calculated using GraphPad Prism.

Table 1
Mean ± S.E.M. tissue weights and responses to methacholine and KPSS[a] in female mouse bladder

Group	*n*	Tissue weight (mg)	pD_2 methacholine	y_{max} (g)	Response to KPSS (g)
A, untreated	20[b]	18.39 ± 0.90	5.43 ± 0.07	2.50 ± 0.29	2.33 ± 0.33
B, 24 h treated	20[b]	17.49 ± 0.74	5.39 ± 0.07	2.59 ± 0.22	2.48 ± 0.029
C, 96 h treated	8	20.13 ± 0.49	5.67 ± 0.04	4.71 ± 0.36[c,d]	4.30 ± 0.42[c,d]
D, pregnant	10	25.00 ± 2.13[c,d]	5.39 ± 0.05	5.89 ± 0.35[c,d]	5.27 ± 0.45[c,d]

[a] High potassium containing Krebs–Henseleit solution.
[b] Data from antagonist control experiments only.
[c] Significantly different from corresponding value for untreated (one-way ANOVAs, Tukey–Kramer, $P<0.01$).
[d] Significantly different from corresponding value for 24 h treated (one-way ANOVAs, Tukey–Kramer, $P<0.01$).

3. Results

3.1. Vaginal smears

Vaginal smears were used to establish histologically cycle stage in untreated mice and oestrogen dominance in oestrogen-treated mice. Two examiners, with 98% concordance, assessed smears from each animal and confirmed oestrogen dominance in 95% of 24 h oestrogen-treated mice ($n=78$). Oestrogen dominance was confirmed in 88% of 96 h oestrogen-treated mice ($n=8$). Of the untreated mice ($n=77$), 8% were in proestrus, 62% in oestrus, 23% in metoestrus, and 5% were in dioestrus. Despite the high proportion of untreated mice in oestrus, which displays similar histology to that of the oestrogen-dominated state, the bolus administration of oestrogen given to groups B and C ensured that circulating oestrogen levels would be much higher than during normal oestrus.

3.2. Tissue weights

Tissue weights are shown in Table 1. Those from pregnant animals were significantly greater than those from untreated and 24 h oestrogen-treated mice ($P<0.01$).

3.3. Radioligand binding studies

Binding curves were routinely analysed to determine whether a one-site fit was most appropriate, and in all cases a one-site fit was indicated. B_{max} estimates for membrane preparations from untreated (group A) and 24 h oestrogen-treated (group B) mice were 88.81 ± 17.88 fmol/mg tissue ($n=4$) and 74.67 ± 7.02 fmol/mg ($n=3$), respectively. K_d values for preparations from untreated and oestrogen-treated animals were 46.22 ± 6.04 and 44.44 ± 4.99 pM, respectively. Student's unpaired t-test indicated no statistically significant differences ($P>0.05$) in either B_{max} or K_d estimates in the two groups, indicating that 1-day oestrogen treatment produced no significant effect upon the binding of the radioligand to bladder homogenate.

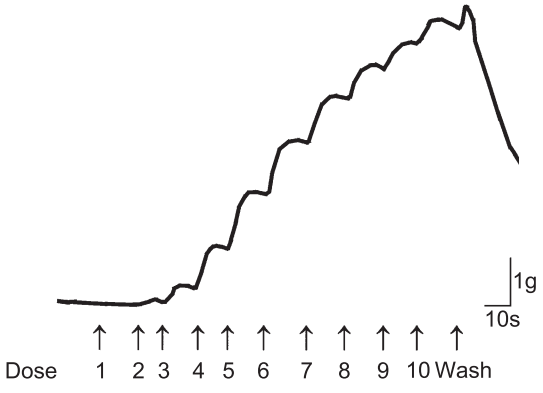

Fig. 1. Trace illustrating cumulative concentration response curves for methacholine producing contractions of a tissue from an untreated mouse (group A) at concentrations from 10 nM (Dose No. 1) to 300 μM (Dose No. 10), shown as additions of log molar concentrations with 0.5 log molar increments.

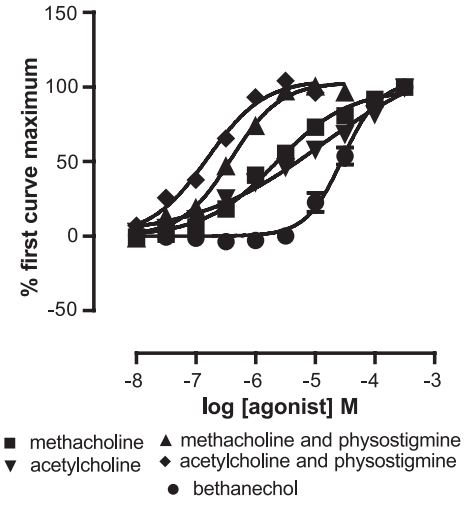

Fig. 2. Mean log concentration–response curves to muscarinic receptor agonists: methacholine ± physostigmine (10 μM, $n=4$), acetylcholine ± physostigmine (10 μM, $n=4$) and bethanechol ($n=4$) ± S.E.M. in tissues from untreated mice. Where not seen S.E.M. are contained within symbols. Data are from time control experiments from first curves and expressed as a percentage of the maximum response.

3.4. Functional studies

3.4.1. Responses to methacholine

All preparations exhibited a stable base line. Methacholine caused concentration-dependent contractions in preparations from all treatment groups. Fig. 1 is a trace showing these effects in a tissue from an untreated mouse (group A). There were no significant differences in the pD_2 values for methacholine in any of the treatment groups, however maximum responses to methacholine were significantly greater in tissues taken from mice 96 h after oestrogen treatment (group C) or from pregnant animals (group D) (Table 1). There was also an increase in the response to KPSS in tissues from each of these groups (Table 1).

3.4.2. Responses to other muscarinic receptor agonists

In a subset of experiments the effects of acetylcholine in the presence and absence of the cholinesterase inhibitor

Table 2
Potencies (mean pD_2/pEC_{50}[a] ± S.E.M.) of muscarinic receptor agonists in untreated and 24 h oestrogen-treated mice

Agonist	n	Untreated	24 h oestrogen-treated
Acetylcholine	4	4.85 ± 0.16[a]	4.83 ± 0.06[a]
Acetylcholine plus physostigmine	4	6.66 ± 0.11	6.97 ± 0.07
Methacholine	20	5.43 ± 0.07	5.39 ± 0.07
Methacholine plus physostigmine	4	6.47 ± 0.06	6.40 ± 0.04
Bethanechol	4	4.57 ± 0.07	4.75 ± 0.08

[a] pEC_{50} = negative logarithm of concentration producing 50% of the maximum response observed.

Table 3
Mean pK_b ± S.E.M. values for muscarinic antagonists versus methacholine on bladder from untreated and 24 h oestrogen-treated female mice compared with published values for male bladder

Antagonist	Untreated	24 h oestrogen-treated	Male[a]
Atropine	9.06 ± 0.16 (8)	9.36 ± 0.17 (8)	9.22
4-DAMP	8.56 ± 1.02 (8)	8.60 ± 0.09 (8)	8.42
HHSiD[b]	7.62 ± 0.06 (6)	7.72 ± 0.08 (6)	N/D
p-F-HHSiD	7.12 ± 0.08 (10)	7.47 ± 0.10 (8)	7.48
Pirenzepine	7.27 ± 0.10 (4)	7.34 ± 0.21 (4)	6.85
Himbacine	6.43 ± 0.21 (4)	6.57 ± 0.06 (8)	N/D
Methoctramine[b]	<7(6)	<7(6)	5.96

N/D, not determined; n = number of estimates.
[a] Data from Choppin and Eglen (2001).
[b] Data from Paravicini et al. (2000).

physostigmine (10 μM), of methacholine in the presence of physostigmine (10 μM), and of bethanechol, were tested in tissues from untreated and 24 h treated mice. Fig. 2 shows the log concentration curves to the agonists in tissues from untreated mice, and Table 2 the pD_2/pEC_{50} values for the agonists in tissues from untreated mice and mice 24 h after administration of oestrogen. Oestrogen treatment did not lead to significant differences in the potencies or maximum responses to any of the agonists (P>0.05) The maximum effects of bethanechol (5.02 ± 0.48 g, n = 21 in tissues from untreated mice and 4.58 ± 0.40 g, n = 16 in tissues from treated mice) were significantly greater than those of methacholine in both groups of animals (P < 0.05), but when expressed as a percentage of the KPSS responses obtained in individual experiments these differences were not significant.

3.4.3. Antagonist studies

Methacholine was chosen as the agonist in the antagonist studies that were conducted using tissues from untreated (group A) and 24 h oestrogen-treated mice (group B). Its effects were competitively antagonised by atropine, 4-DAMP, *p*-F-HHSiD, pirenzepine, and himbacine in a concentration-dependent manner. Vehicle for *p*-F-HHSiD (ethanol 0.1% and 1%) were without effect on methacholine concentration–response curves (P>0.05), and time control experiments indicated no significant changes in pD_2 values for the agonist. Mean antagonist potencies (pK_B) for each experimental group ± S.E.M. are shown in Table 3. The rank order of potency of muscarinic antagonists was as follows: atropine ≥ 4-DAMP>*p*-F-HHSiD ≥ pirenzepine>himbacine; there were no significant differences in the potencies of any one of the antagonists in the two treatment groups. Fig. 3 shows correlation plots of pK_d values versus methacholine in the female urinary bladder and the pK_i estimates for these muscarinic antagonists at human recombinant muscarinic receptors obtained in radioligand binding studies (Dorje et al., 1991). There were significant correlations (P < 0.05) for both untreated and 24 h oestrogen-treated mice at muscarinic m_1 and m_5 receptors, but poor correlations at muscarinic m_2 and m_4 receptors. Correlations at musca-

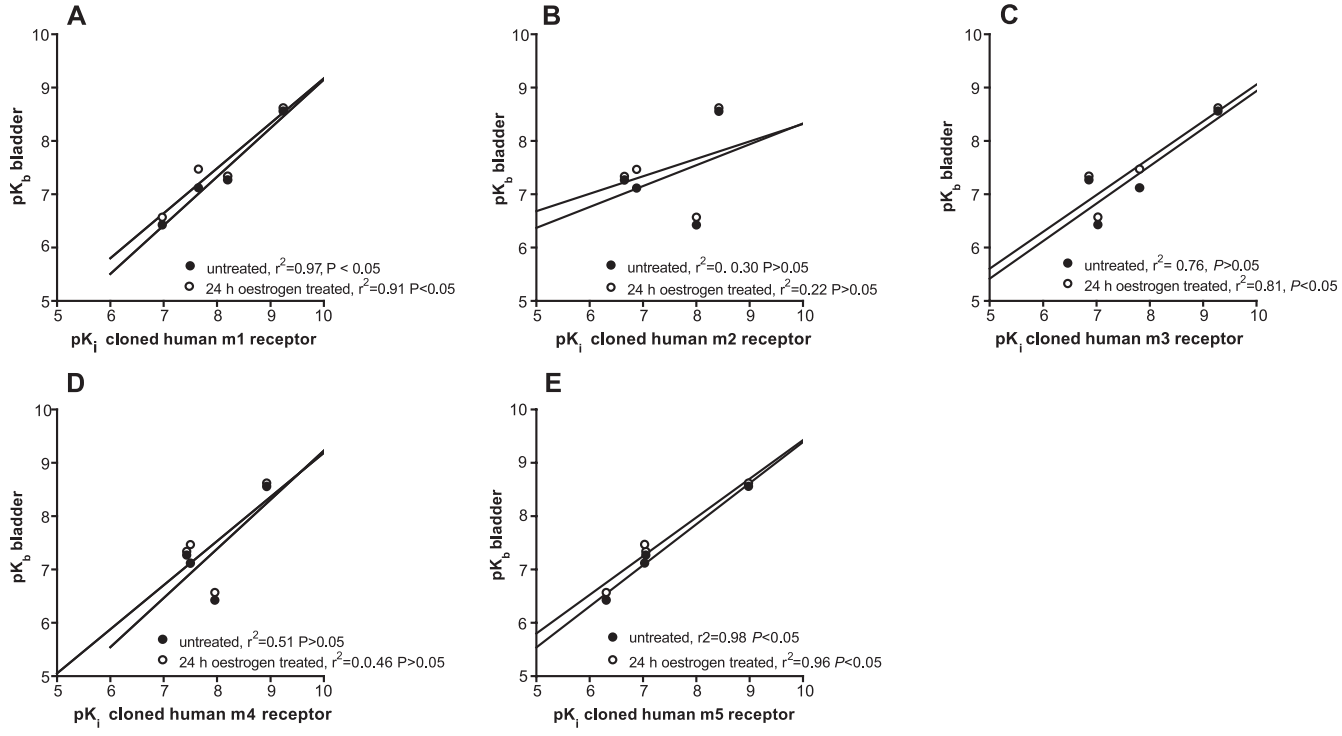

Fig. 3. Correlation plots between the pK_b values for antagonists versus methacholine at muscarinic receptor mediating contraction in female mouse bladder and pK_i values obtained at human recombinant muscarinic m_1 to m_5 (A–E) receptors. The binding data were from Dorje et al. (1991). r^2 values were from Pearson correlation analyses. Lines indicate the correlation plots.

rinic m_3 receptors were significant (group A, $r^2 = 0.66$), (group B, $r^2 = 0.77$) only if the estimates obtained previously (Table 3) by Paravicini et al. (2000) were included in the correlation. For this correlation, the pK_i estimates for HHSiD at muscarinic m_3 receptors were from Buckley et al. (1989).

The pK_b estimate for atropine versus methacholine in bladders from pregnant mice was 9.36 ± 0.12 ($n = 6$).

4. Discussion

The aim of this project was to investigate the numbers and subtype/s of muscarinic receptors mediating female mouse bladder contraction. A secondary objective was to investigate the effects of the in vivo administration of oestrogen on responses mediated by these receptors, since the effect of oestrogen and its mechanism on the bladder is still unclear.

Functional studies indicated that, as in the male bladder (Choppin and Eglen, 2001), muscarinic receptor agonists were generally of low potency in causing detrusor contraction. In the case of acetylcholine and methacholine, this was in part due to inactivation by cholinesterases, as the potencies of both these agonists were increased approximately tenfold in the presence of the cholinesterase inhibitor, physostigmine. Our finding that the potencies of the agonists were unchanged by 24 h oestrogen treatment is consistent with the data from the saturation radioligand binding studies that indicated that muscarinic receptor affinities and densities were similarly unaffected by this treatment regime.

Antagonist affinities were not investigated in our radioligand binding studies. In general, however, the antagonist potencies and their rank order estimated in the present functional studies (see Table 3) are compatible with the notion that as in the male, the muscarinic receptor/s mediating mouse bladder contraction in the female are of the muscarinic M_3 subtype. Thus the mean pK_b values, in tissues from untreated and 24 h treated mice, for atropine (9.06, 9.36), 4-DAMP (8.56, 8.60), *p*-F-HHSiD (7.12, 7.5) pirenzepine (7.27, 7.34), himbacine (6.43, 6.57), taken together with previous estimates for HHSiD (7.62, 7.72) and methoctramine (<7) (Paravicini et al., 2000), are broadly consistent with literature estimates implicating muscarinic M_3 receptors (Choppin and Eglen, 2001; Choppin, 2002) for such activation. However, as in the earlier report with the male mouse (Choppin and Eglen, 2001), we obtained an excellent correlation between the potencies of the antagonists studied and those described for cloned human muscarinic m_5 receptors, as indicated by the correlation plot shown in Fig. 3. There was also good correlation with dissolation constants obtained at cloned human muscarinic m_1 receptors. Involvement of muscarinic M_2 and M_4 receptors in mediating detrusor contraction was clearly excluded bu our data.

In vitro studies in other species (rat, guinea-pig and human) implicate the muscarinic M_3 receptor as mediating cholinoceptor-induced contraction (Chess-Williams et al., 2001; Fetscher et al., 2002; Hegde et al., 1997; Wang et al., 1995). Our data excluding the notion the activation of a muscarinic M_2 receptor mediates female mouse bladder detrusor contraction are of interest in the context of reports of the presence of high numbers of muscarinic M_2 muscarinic receptors in mammalian bladder from most species. Thus immunological and molecular studies indicate that the muscarinic M_3 receptors constitute only 10–30% of the muscarinic receptor population of rat bladder which was reported to consist of only muscarinic M_2 and M_3 receptors (Braverman et al., 1998a,b). In the human also muscarinic M_3 receptors comprise only a small proportion of the subtypes present in the bladder (Chess-Williams et al., 2001).

It has recently been reported that in male and female mice lacking the gene for the muscarinic M_3 receptor subtype the detrusor muscle response to carbachol was reduced (Matsui et al., 2000). In this knockout mouse the residual response to this agonist was attributed to activation of muscarinic M_2 receptors (Matsui et al., 2000). More recently, Matsui et al. (2002) reported that, in their hands, carbachol was ineffective in contracting the bladder of female mice lacking both muscarinic M_2 and M_3 acetylcholine receptors. They consequently proposed that detrusor contraction is mediated by non-cholinergic mechanisms in such mice, since bladder function in the female remained apparently normal. However, a substantial cholinergic component remained in response to field stimulation of intramural nerve terminals in bladders from these mice (Matsui et al., 2002). An alternative possibility, suggested by our observations, is that muscarinic M_1 and/or M_5 receptors may also play a role in mediating detrusor contraction in the female mouse. In this case, however, it would be necessary to propose that carbachol is a relatively poor agonist for these receptors. Carbachol was investigated in two preliminary experiments in the present study, in our hands it was less potent than methacholine (data not shown). Matsui et al. (2000, 2002) did not investigate any other muscarinic receptor agonists other than carbachol in their studies of knockout mice.

An interesting finding of the present study was that 96 h after oestrogen treatment there was an increase in the maximum response of the female mouse bladder to methacholine. This increase was, however, accompanied by an increase in the response to KPPS, indicating a non-specific effect of oestrogen on bladder contractility. Levin et al., (1980) using female rabbit bladder, observed results similar to those observed in the present study. In that study, a single injection of oestrogen led to an increase, 4 days later, in the contractile response of female rabbit bladder to carbachol in vitro. However, with more prolonged (3 weeks) treatment (Shapiro, 1986) a significant decrease in muscarinic receptor density was reported. These findings suggest that oestrogen

treatment may initially have a dual effect, increasing contractile response to muscarinic receptor agonists several days after treatment, then progressively decreasing this after more prolonged treatment. Further studies are clearly indicated, given the equivocal effect of oestrogen and combined hormone replacement therapy in managing urinary incontinence in postmenopausal women (Miodrag et al., 1988).

In conclusion, the female mouse urinary bladder exhibits a broadly similar profile of muscarinic receptor activation to that in the male and clearly has potential as an animal model for studying human female bladder disorders. Although short-term treatment with oestrogen can clearly influence bladder contractility, we obtained no evidence for a specific effect of such treatment on muscarinic receptor function.

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