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Different responses to drugs against overactive bladder in detrusor muscle of pig, guinea pig and mouse

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Abstract

Direct comparison of experimental data for drugs commonly used in the treatment of overactive bladder is difficult because of possible species differences. In this study, we compare the effects of atropine, propiverine, oxybutynin and tolterodine in strips of pig, guinea pig and mouse detrusor muscle. In the three species, we observed slight differences in potency of carbachol-induced biphasic contractile responses between the species (guinea pig>pig>mouse). Cumulative concentration–response curves for carbachol were shifted to the right by atropine, propiverine, oxybutynin and tolterodine. However, at higher concentrations of the latter three antagonists, the maximum response to carbachol was also reduced. Therefore, propiverine, oxybutynin and tolterodine must have additional pharmacological actions beyond competitive antagonism at muscarinic receptors. Electric field stimulation (30 Hz) of detrusor strips led to contraction amplitudes, which remained constant over time (210 min) in pig, decreased by 17 ± 5% in guinea pig, and increased by 28 ± 9% in mouse detrusor muscle. Electric field stimulation-evoked contractions were suppressed to 18% of pre-drug control by high concentrations of atropine (10 μM) in pig, but to a much lesser extent in guinea pig and mouse (to 46% and 70%, respectively). In all three species, a myogenic component of contraction was observed in the presence of tetrodotoxin (1 μM). Compared to atropine, the bladder spasmolytic agents propiverine, oxybutynin and tolterodine also reduced electrically evoked contractions in the three species, though higher concentrations were required. The differences in the reported effects of the spasmolytic agents commonly used for treating overactive bladder suggest that drug action is strongly dependent on the species. Thus, a comparison of drug effects is only feasible in the same animal model and the results cannot easily be transferred to humans.

Keywords: Detrusor smooth muscle; Electrical field stimulation; Atropine; Propiverine; Oxybutynin; Tolterodine

1. Introduction

The effectiveness of drug treatment of bladder instability is only moderate (Andersson, 2000) and many of the antimuscarinic drugs currently on the market for this indication have side effects that limit their clinical usefulness (de Groat and Yoshimura, 2002; Turner and Brading, 1997). Development of new agents for treating the overactive bladder requires a sound understanding of detrusor function as well as reliable in vitro models for comparison of novel compounds with commonly used drugs (Sellers et al., 2001; Yono et al., 2000).

In mammals, contractile responses in the urinary bladder preparations can be induced by several mechanisms: (i) stimulation of the parasympathetic nerves, (ii) field stimulation of intrinsic excitatory nerves or smooth muscle cells, (iii) direct application of acetylcholine to the muscle strips (Brading and Mostwin, 1989). The responses evoked by electrical field stimulation of preparations from nonprimate bladders are partially resistant to blockade with atropine (Andersson et al., 2001; Brading and Williams, 1990; Sibley, 1984). This nonadrenergic, noncholinergic mechanism (NANC) is probably mediated by ATP (Burnstock et al., 1978; Ralevic and Burnstock, 1998; Tong et al., 1997). The relative contribution of cholinergic and NANC mechanisms for nerve-mediated muscle contractions vary in different species with a choliner-

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gic mechanism predominating in pig and human bladders (Sibley, 1984) and a NANC mechanism predominating in small mammals (Pessina et al., 2001). Under some pathological conditions, however, the NANC mechanism may become of significance even in pigs and humans (Bayliss et al., 1999; Palea et al., 1993; Sibley, 1984; Sjögren et al., 1982). Nevertheless, the main part of detrusor muscle contraction in the hyperactive human bladder is considered to be mediated via stimulation of muscarinic receptors (Andersson, 1993; Chapple, 2000). Tolterodine, for instance, has been introduced as a muscarinic receptor antagonist intended for treatment of symptoms related to an overactive bladder (Clemett and Jarvis, 2001; Nilvebrant et al., 1997; Wefer et al., 2001; Yono et al., 2000). In contrast, propiverine has been described to affect calcium influx and calcium homeostasis in addition to its antimuscarinic action (Haruno, 1992; Madersbacher and Mürtz, 2001). Oxybutynin mainly acts as an antimuscarinic compound but also directly relaxes smooth muscle (Andersson and Chapple, 2001). However, much of the pharmacology of spasmolytic agents is derived from in vitro studies on detrusor smooth muscle from various animal species. Therefore, a direct comparison of available data is difficult due to species differences (Levin et al., 1994; Mersdorf et al., 1993).

In this work, we systematically compared the effects of the anticholinergic drugs atropine, propiverine, tolterodine and oxybutynin on contractile tension in detrusor muscle strips of the urinary bladder of three species. The pig bladder was chosen because of its similarity to the human bladder with respect of the NANC parts of contractility (Sellers et al., 2000; Sibley, 1984). For comparative purposes, guinea pig and mouse urinary bladder were studied. The mouse species was chosen in particular because of its common use as a transgenic animal.

Contraction properties of the detrusor muscles were characterized by concentration–response curves for carbachol and by frequency–response curves elicited with electric field stimulation in the three chosen species. The aim of our study was to create comparative data for the three animal models—pig, guinea pig and mouse detrusor smooth muscle—which are necessary for assessing data of future substance testing.

2. Materials and methods

2.1. Preparation of detrusor muscle strips

Urinary bladders of female pigs were obtained from a local abattoir. For transportation, the bladders were immediately placed in modified Ringer solution at 4 °C. For the preparation of suitable muscle strips, the bladder trigone was removed from the rest of the bladder because of known functional differences between the bladder trigone and the bladder wall (Pagala et al., 2001). A 2 × 2 cm slice was cut off from the anterior wall. After removing the serosa and mucosa, four longitudinal muscle strips (7–10 mm length and 2–4 mm width) were cut from the bladder wall.

Male guinea pigs, weighing 250–400 g (Dunkin Hartley strain from Charles River) were anaesthetised and sacrificed by cervical dislocation. The urinary bladder was removed at the bladder neck. After cutting off the dome of the bladder, the remaining muscle ring was opened longitudinally. The mucosal layer was removed and four strips of smooth muscle around 5–8 mm long and 2 mm wide were prepared.

Male C57Bl6 mice (Charles River) weighing 20–30 g were sacrificed by cervical dislocation. Preparation of the urinary bladder was similar as in the guinea pig, but due to its small size only two strips of 2–3 mm length and 1 mm width were obtained.

2.2. Detrusor muscle contraction experiments

The tissues were mounted in 25-ml organ baths containing carbogen-gassed Tyrode's solution, which was maintained at 37 °C. The isometric tension generated by the tissues was measured with an isometric force transducer (GM 2, Föhr Medical Instruments, Seeheim/Ober Beerbach, Germany), amplified and recorded with Chart 4.0™, a data and recording system provided by ADInstruments (Sydney, Australia). The resting load was 10 mN and was repeatedly adjusted during the equilibration period of 60 min. The strips were exposed twice for 15 min to carbachol (1 μM in pig or 1 and 10 μM in guinea pig and mouse) with a period of 15 min of washout between the two treatments. After an additional equilibration period of 20 min without any further mechanical manipulation, the muscle strips were either stimulated with carbachol or subjected to electrical field stimulation.

Following the equilibration period, noncumulative concentration–response curves of carbachol were obtained from 0.01 to 100 μM in guinea pig and pig or from 0.1 to 1000 μM in mouse muscle strips. The exposure time to each concentration was 15 min with 2–10 min of washout between two additions of carbachol. This protocol was used to investigate the phasic and the tonic responses to carbachol in pig, guinea pig and mouse detrusor muscle strips.

In addition, the anticholinergic actions of atropine, propiverine, tolterodine and oxybutynin were studied by measuring their effects on cumulative concentration–response curves for carbachol in separate experiments. Using this protocol, carbachol was added in cumulative increasing concentrations starting with 30 nM (increments of half log unit) at the end of the equilibration period. After a washout period of 1 h, the test drug was added and the second concentration–response curve for carbachol was started after one further hour in the presence of the test compound. Peak increase of force induced by the individual carbachol concentration is expressed the percentage of the maximum effect observed during the first concentration–response curve. For comparative purposes, time-matched control experiments (TMC) were run without any drug added. At least three to seven experiments with bladder strips from

three to seven different animals were performed for each concentration of the antagonist.

The frequency–response curves were run from 2 to 50 Hz (2, 4, 8, 10, 16, 20, 30, 40, 50 Hz) with 10 successive trains of rectangular impulses (1 ms pulse duration) at each frequency (stimulator, Föhr Medical Instruments). Train duration was 2 s (mouse), 4 s (guinea pig) or 5 s (pig) with 2 min intervals. The applied current was 60–90 mA. To test the effects of either atropine, propiverine, tolterodine or oxybutynin (0.1 nM–1 mM), the muscle strips were stimulated with 30 Hz. After constant contraction amplitudes were reached (approximately after 30 min), increasing concentrations of the respective substances were added in a cumulative manner (exposure time of 30 min each). At the end of each experiment, tetrodotoxin (1 μM) was added to quantify the nerve-mediated component of the contraction.

2.3. Drugs

The modified Ringer's solution contained (in mM): 149 NaCl, 2.7 KCl, 1.8 $CaCl_2$, 0.1 NaH_2PO_4, 0.7 Na_2HPO_4, 5.6 glucose. The Tyrode's solution contained (in mM): 127 NaCl, 5.4 KCl, 1.05 $MgCl_2$, 1.8 $CaCl_2$, 0.4 NaH_2PO_4, 22 $NaCO_3$, 5.6 glucose. All chemicals were of analytical grade and purchased from Sigma-Aldrich (Taufkirchen, Germany). Carbachol, atropine sulphate, oxybutynin-HCl and tetrodotoxin acetate were also purchased from Sigma-Aldrich. (*R*)-Tolterodin-L (+)-tartrate and propiverine-HCl were provided by Apogepha Arzneimittel, Dresden, Germany. Drugs were made up as 0.1 M stock solutions in Milli-Q-water (carbachol), acetate buffer (tetrodotoxin) or dimethyl sulfoxide (DMSO; all anticholinergics) and further diluted with Milli-Q-water.

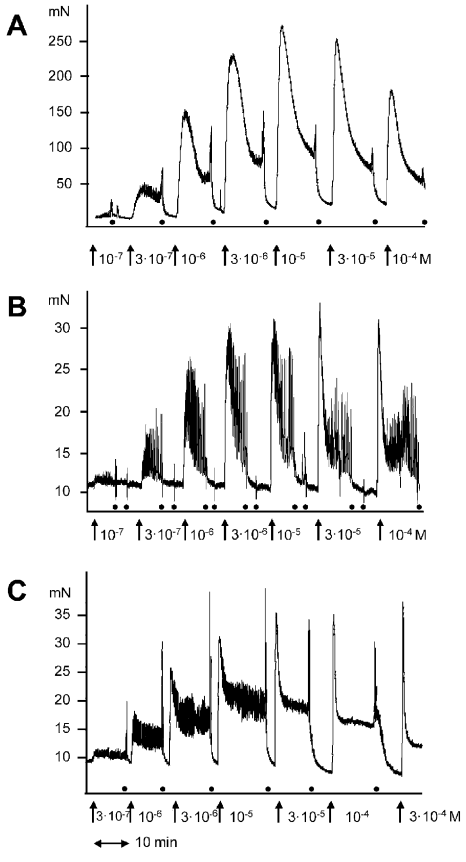

Fig. 1. Representative original recordings of contractile responses of detrusor strips from pig (A), guinea pig (B) and mouse (C) during exposure to increasing concentrations of carbachol. Exposure time was 10–20 min with 2–10 min of washout before the next concentration was added. The resting force was adjusted to ~10 mN in all three species. Arrows symbolise the time of carbachol addition, dots indicate washout.

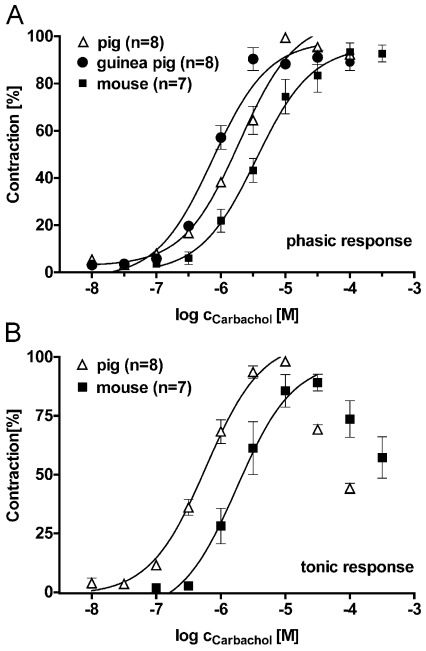

Fig. 2. Concentration–response curves for carbachol in detrusor muscle strips from pig, guinea pig and mouse. Phasic (A) and tonic (B) responses are shown in percent of the maximum response in each individual muscle strip. Curves were fitted without the declined values in force of contraction at higher concentrations. In mouse and pig detrusor strips, tonic responses were analysed at average force development 2 min before washout. For guinea pig strips, tonic responses could not be analysed because of the extensive and irregular spontaneous activity. The data are shown as means ± S.E.M. of *n* experiments.

2.4. Data analysis

Responses to the carbachol stimulation are expressed as a percentage of the maximum force induced by carbachol. In addition to the maximum phasic contraction, carbachol-induced tonic responses were also analysed in the pig and mouse detrusor strips. Differences between the tonic responses to carbachol in pig and mouse were analysed for significance using Student's *t*-test for unpaired variates.

Cumulative concentration–response curves were analysed by nonlinear regression of each individual experiment using GraphPad Prism® 3.02 (GraphPad Software, San Diego, USA), mean EC_{50} values (molar carbachol concentration producing 50% of the maximum contraction response)

were calculated for concentration–response curves before and after test drug addition.

Average values for the electrically induced muscle contraction amplitudes were obtained from the last five contractions before the next concentration increase. All data are expressed as the mean ± S.E.M. (standard error of the mean). The magnitude of drug effect is given as the percentage of inhibition of the electrically evoked contraction amplitude before any substance addition (= 100%). Individual IC_{50} (molar drug concentrations producing 50% inhibition of maximum contractile response to electric field stimulation) were determined by nonlinear regression analysis for each individual experiment using GraphPad Prism® 3.02 (GraphPad Software). Mean IC_{50} values were calculated from n experiments for one tested drug. These values are presented as $-\log IC_{50}$ (mean ± S.E.M. of n experiments) in Table 1. To evaluate differences in the effects of all compounds investigated on the electrically induced contractions, one-way analysis of variances (ANOVA between groups) with an additional Bonferroni's multiple comparison test was performed and differences were considered significant at P-values < 0.05. All experiments were repeated at least five times on muscle strips originating from at least three different animals. The highest concentration of DMSO as solvent (1% vol/vol) produced no significant effects on the maximum muscle contraction.

3. Results

3.1. Carbachol concentration–response curves

Increasing concentrations of the muscarinic receptor agonist carbachol (0.1–100 μM)-induced contractile responses in isolated detrusor muscle strips from the three different species we have chosen to investigate (Fig. 1).

Force development initially increased to a transient peak (phasic response) and then declined to reach a new plateau level (tonic response) before washout. This general pattern was superimposed by spontaneous force oscillations of varying intensity depending on species. In guinea pig detrusor strips, the spontaneous activity was so extensive that it prevented analysis of the tonic carbachol response.

The maximum force values obtained with 100 μM carbachol were: 89 ± 18 mN in the pig ($n = 12$), 32 ± 4 mN in the guinea pig ($n = 12$) and 9 ± 2 mN in the mouse detrusor muscle ($n = 12$). When corrected for wet weight (ww) of the muscle strips, the values were 0.5 ± 0.1 ($n = 12$) mN/mg ww (pig), 6.2 ± 0.9 ($n = 12$) mN/mg ww (guinea pig) and 2.6 ± 0.6 ($n = 12$) mN/mg ww (mouse).

The concentration dependence of the carbachol effects (Fig. 2) yielded $-\log EC_{50}$ (M) values for the phasic responses of 5.68 ± 0.05 for pig, 6.12 ± 0.07 for guinea pig and 5.45 ± 0.11 for mouse detrusor, respectively. One-

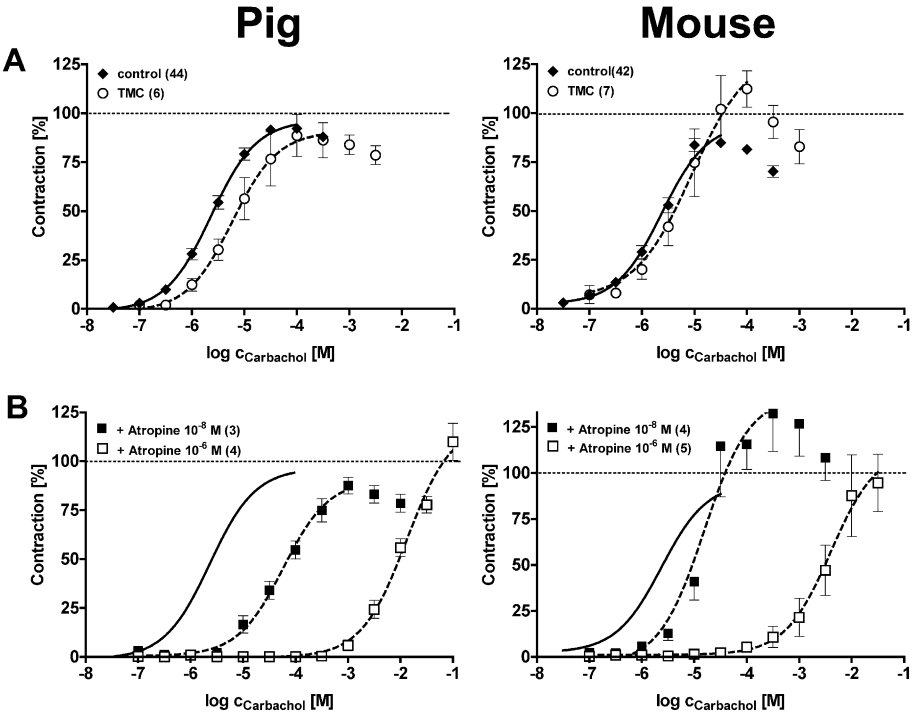

Fig. 3. Effect of atropine on cumulative concentration–response curves for carbachol (30 nM–10 mM) in comparison to time-matched control experiments (TMC) in detrusor muscle strips of pig and mouse. Data are shown as means ± S.E.M. of n experiments. Responses to carbachol are expressed in percent of the maximum effect in the first concentration–response curve. Note that the continuous and dashed lines have been fitted to the mean values without considering the decline in force at very high concentrations. Control data in (A) (continuous line) are averaged values from the first concentration–response curves in all experiments (pig $n = 44$; mouse $n = 42$) and are also depicted in (B) without data points. (A) Time-matched control experiments without any drug added; (B) effects of atropine on the concentration–response curve of carbachol.

way ANOVA showed a significant difference between the guinea pig and the pig ($P<0.01$), and between guinea pig and mouse detrusor ($P<0.001$). The $-\log EC_{50}$ values for the tonic responses to carbachol stimulation in pig and mouse were 6.22 ± 0.07 and 5.58 ± 0.15, respectively ($P<0.01$, Student's *t*-test).

3.2. Effects of the spasmolytic agents on cumulative concentration–response curves for carbachol

For comparison of the anticholinergic actions of the tested drugs, cumulative concentration–response curves were studied with 10 min of exposure to each carbachol concentration. Due to the strong increase of myogenic contractions with carbachol in guinea pig detrusor strips, only preparations from pig and mouse could be used. In each muscle strip, an initial concentration–response curve served as the internal control of responsiveness to carbachol and the second concentration–response curve was obtained after 1 h of incubation with the test drug, the results are depicted in Figs. 3 and 4.

In pig detrusor muscle strips, the maximum force increase in the control concentration–response curve was 0.7 ± 0.1 mN/mg wet weight ($n=44$), the mean value $-\log EC_{50}$ was 5.7 ± 0.1 ($n=44$). Atropine shifted the concentration–response curve to the right without any suppression of the maximum response as expected for a pure competitive muscarinic antagonist (Table 1). Propiverine, oxybutynin and tolterodine also shifted the carbachol concentration–response curve to the right, however, these drugs additionally suppressed the maximum of the curves at higher concentrations. In the concentration range tested, oxybutynin and tolterodine caused larger shifts of the concentration–response curve and larger depressions of the maximum response than propiverine.

In the mouse detrusor, the maximum force increase in the control concentration–response curve was 2.24 ± 0.3 mN/

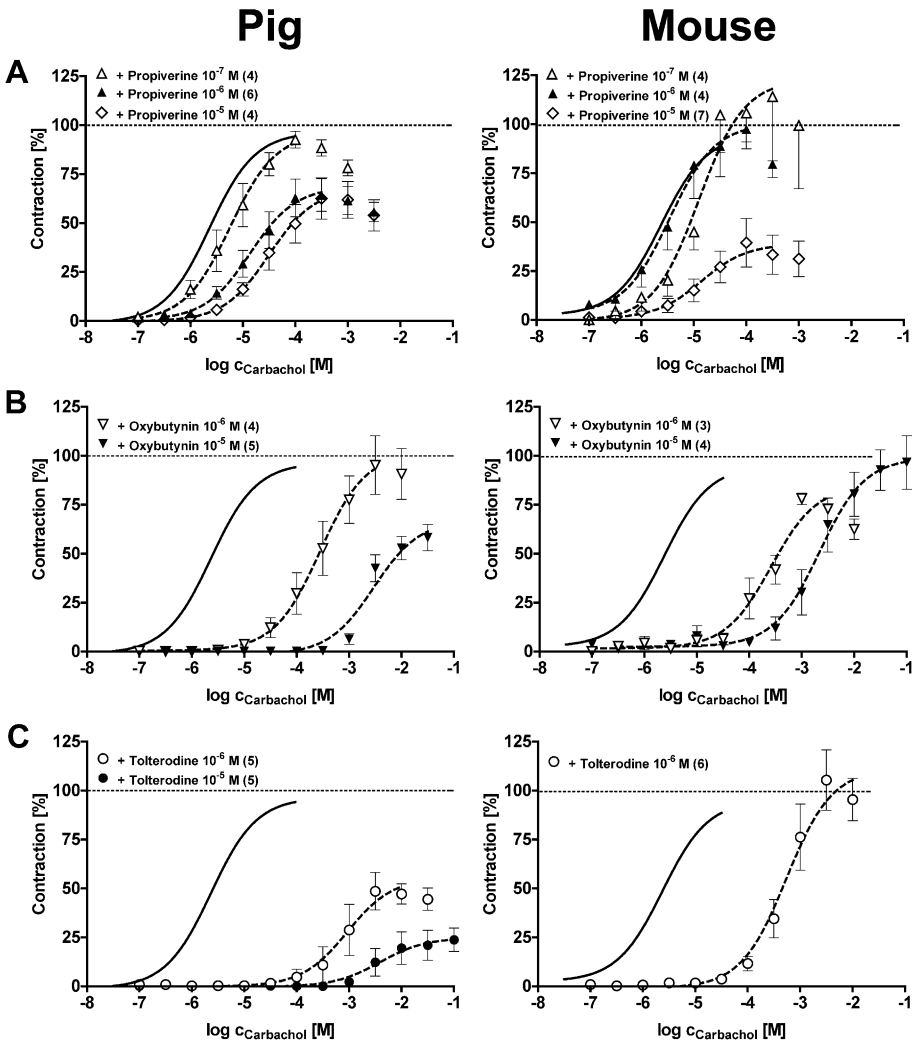

Fig. 4. Effects of spasmolytic drugs on cumulative concentration–response curves for carbachol (30 nM–10 mM) in detrusor muscle strips of pig and mouse. Data are shown as means ± S.E.M. of *n* experiments. Responses to carbachol are expressed in percent of the maximum effect in the first concentration–response curve (continuous lines, mean curves as in Fig. 3). (A) Effects of propiverine, (B) effects of oxybutynin and (C) effects of tolterodine on the concentration–response curve of carbachol.

Table 1
Responses to carbachol before and after addition of test compounds

Compound	Concentration (M)	n	$-\log EC_{50}$; carbachol		$\Delta -\log EC_{50}$	Eff_{max} (%)
			First CRC	Second CRC		
(A) Pig						
TMC	–	6	5.27 ± 0.18	4.98 ± 0.19	0.29 ± 0.07	91 ± 8
Atropine	10^{-8}	3	5.66 ± 0.17	4.28 ± 0.15	1.38 ± 0.02***	87 ± 4
	10^{-6}	4	5.70 ± 0.10	1.91 ± 0.03	3.79 ± 0.10***	110 ± 7
Propiverine	10^{-7}	4	5.54 ± 0.25	5.18 ± 0.18	0.36 ± 0.18	92 ± 4
	10^{-6}	6	5.50 ± 0.11	4.78 ± 0.16	0.72 ± 0.13	64 ± 8*
	10^{-5}	4	5.53 ± 0.32	4.42 ± 0.12	1.12 ± 0.23*	62 ± 11*
Oxybutynin	10^{-6}	4	5.64 ± 0.16	3.51 ± 0.17	2.15 ± 0.12***	95 ± 15
	10^{-5}	5	5.39 ± 0.14	2.51 ± 0.05	2.88 ± 0.12***	58 ± 7**
Tolterodine	10^{-6}	5	6.01 ± 0.29	2.87 ± 0.16	3.13 ± 0.31***	57 ± 9*
	10^{-5}	5	4.99 ± 0.23	1.96 ± 0.25	3.04 ± 0.23***	25 ± 7***
(B) Mouse						
TMC	–	7	5.21 ± 0.14	4.75 ± 0.22	0.48 ± 0.19	112 ± 9
Atropine	10^{-8}	4	5.08 ± 0.29	4.68 ± 0.18	0.05 ± 0.22	132 ± 21
	10^{-6}	5	5.43 ± 0.22	2.70 ± 0.24	2.73 ± 0.35***	95 ± 16
Propiverine	10^{-7}	4	5.41 ± 0.37	4.06 ± 0.27	1.35 ± 0.44	114 ± 33
	10^{-6}	4	5.27 ± 0.10	4.24 ± 0.62	1.05 ± 0.56	103 ± 10
	10^{-5}	7	5.49 ± 0.18	4.66 ± 0.27	0.83 ± 0.26	40 ± 12***
Oxybutynin	10^{-6}	3	5.66 ± 0.12	3.53 ± 0.12	2.13 ± 0.15**	78 ± 3
	10^{-5}	4	5.27 ± 0.04	2.46 ± 0.22	2.82 ± 0.23***	105 ± 6
Tolterodine	10^{-6}	6	5.38 ± 0.21	3.04 ± 0.09	2.34 ± 0.20***	112 ± 13

Cumulative concentration–response curves (CRC) were used for calculation of $-\log EC_{50}$ values in individual experiments before and after exposure to test compound (first and second CRC, respectively). The shift in concentration–response curve is expressed as the difference between $-\log EC_{50}$ values of the first and second CRC ($\Delta -\log EC_{50}$). Maximum effect (Eff_{max}) is expressed in percent of maximum response in the first CRC. Data in mean ± S.E.M.; EC_{50} in mol/l); TMC, time-matched control. *$P<0.05$, **$P<0.01$, ***$P<0.001$ when compared the $\Delta -\log EC_{50}$ and the Eff_{max}-value to those of the time-matched control experiments in a one-way ANOVA with an additional Bonferroni's multiple comparison test.

mg wet weight ($n=42$), the mean value $-\log EC_{50}$ was 5.4 ± 0.1 ($n=42$). Although the scatter of data points was generally larger with mouse than with pig detrusor muscle strips, the responses to the test drugs clearly differed between these two species. While atropine (0.01 and 1 μM) also shifted the concentration–response curve for carbachol to the right without any effect on response maximum, the highest concentration of propiverine depressed the maximum response to a larger extent than in the pig muscle. Oxybutynin and tolterodine, on the other hand, only caused rightward shifts without diminishing the maximum of the curves.

3.3. Electric field stimulation-induced detrusor contractions

The contractile responses to electrical field stimulation depended on frequency with a similar force–frequency relation in pig, guinea pig and mouse (Fig. 5). Typical contractile responses of the pig detrusor muscle at various frequencies are depicted in the inset of Fig. 5, contraction amplitudes increased with frequency to maximum values above 30 Hz. This frequency was chosen for all further experiments.

The stability of electrically induced contractile responses (30 Hz) over time varied between the three species (Fig. 6). In the course of time-matched control experiments, tension development remained constant in pig, decreased in guinea pig and increased in mouse detrusor muscles, the values after 210 min being +5 ± 5%, −17 ± 5% and +28 ± 9% of control, respectively. In the presence of the nerve conduction blocker tetrodotoxin (1 μM), electrically induced tension was substantially reduced in all three species.

Atropine impaired electrically induced tension albeit to a variable extent. Contractions were reduced to 18 ± 2% of

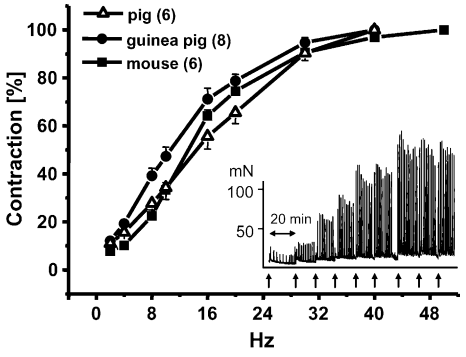

Fig. 5. Tension development of detrusor muscle strips in response to short trains of electrical field stimulation at increasing frequencies. The contraction amplitude is expressed in percent of tension developed at 50 Hz. Stimulation parameters: amplitude, 60–80 mA; single pulse duration 1 ms; duration of train of stimuli, 2–5 s; interval between trains, 2 min. Inset: representative tracings from a pig detrusor muscle strip; arrows indicate frequency of stimulation (from left to right: 2, 4, 8, 10, 16, 20, 30, 40, 50 Hz).

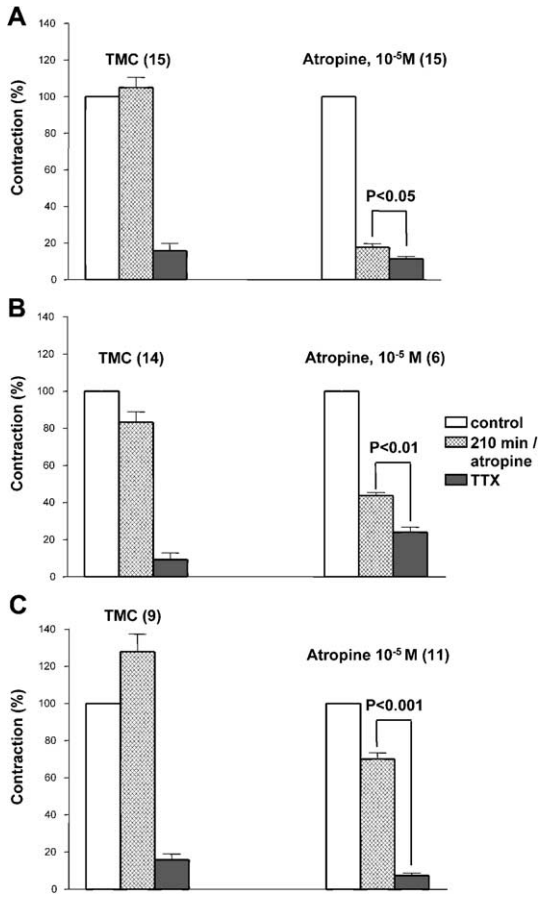

Fig. 6. Changes in electrically stimulated force development of detrusor muscle strips in time-matched control experiments (TMC, left columns) and in the presence of 10^{-5} M atropine (right set of columns). Investigated species: pig (A), guinea pig (B) and mouse (C). Each set of columns represents from left to right: control value at the end of equilibration (white columns), after 210 min of stimulation or in the presence of 10^{-5} M atropine (hatched), and after addition of tetrodotoxin (1 μM) to block the nerve conduction (dark grey). The contraction force is expressed in percent of pre-drug control. Control forces (=100%) of the two groups of muscle strips were similar in each species but varied between species (in mN/mg wet weight of the strips) was in pig: time-matched control 0.32 ± 0.04 ($n=15$), atropine 0.40 ± 0.04 ($n=15$); guinea pig: time-matched control 3.49 ± 0.60 ($n=14$), atropine 2.34 ± 0.54 ($n=6$); mouse: time-matched control 5.43 ± 0.75 ($n=9$), atropine 5.32 ± 0.88 ($n=6$). Values are presented as means ± S.E.M. from n experiments.

pre-drug control in pig detrusor muscle, to $44 \pm 1\%$ in guinea pig and only to $70 \pm 3\%$ in mouse. Additional exposure to 1 μM tetrodotoxin decreased contraction amplitudes further to $11 \pm 1\%$ in the pig, to $24 \pm 3\%$ in the guinea pig and to $7 \pm 1\%$ in the mouse. These results suggest that electric field stimulation-evoked tension is mainly cholinergic in pig but has a large noncholinergic component in the other two species. The fraction of tension persisting in the presence of 10 μM atropine was defined as atropine-resistant tension.

Concentration–response curves of the investigated bladder spasmolytic drugs were constructed from the effects with increasing concentrations with 30 min of exposure time between concentration increments. The stability of the muscle strip preparations in time-matched controls without any drug addition and the effects of atropine are shown in Fig. 7 for pig and mouse detrusor.

Fig. 8 summarizes the complete concentration–response curves for all investigated spasmolytic drugs, i.e. atropine, propiverine, tolterodine and oxybutynin and also includes time-matched controls. The respective IC_{50} values in M are shown in Table 2 expressed as the $-\log IC_{50}$.

In the pig detrusor muscle, atropine reduced the electrically evoked contractions in significantly lower concentrations compared to the other drugs. Almost three orders of magnitude higher concentrations were required to produce an effect of similar size with propiverine, tolterodine and oxybutynin. The concentration–response curve of propiverine exhibited a steep slope, whereas the respective concentration–response curves of tolterodine and oxybutynin were flatter.

In the guinea pig, atropine reduced the electrically evoked contractions to a lesser extent than in the pig (see Fig. 8), the concentrations required were significantly lower (see Table 2). Very high concentrations of atropine (0.3 and 1 mM) further impaired "atropine-resistant" tension. Using propiverine, the effective impairment of contraction was observed with concentrations above 10 μM, although contraction amplitude also tended to decrease at concentrations in which atropine was effective. This effect was small and difficult to

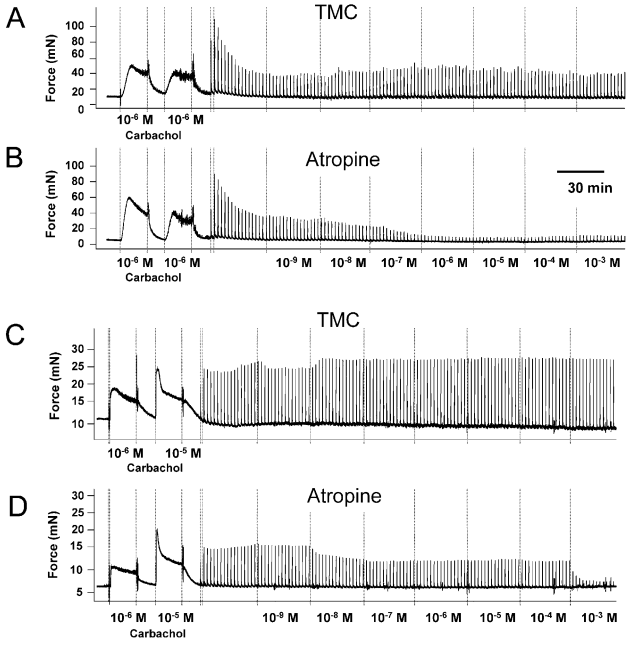

Fig. 7. Representative original tracings of contraction force (mN) development of pig (A, B) and mouse detrusor muscle strips (C, D) in response to electrical field stimulation (30 Hz). (A) and (C), time-matched control (TMC); (B) and (D), effect of increasing atropine concentrations. The responsiveness was tested in each preparation by two exposures to carbachol. Weight of individual muscle strips: 134.3 mg (A), 134.1 mg (B), 2.6 mg (C), 2.2 mg (D).

separate from the slight spontaneous deterioration of tension in the time course of an experiment in these species (see time-matched control in Fig. 8). However, the difference between contraction amplitude in the presence of 1 μM propiverine (25 ± 5% force reduction) and time-matched control experiments (10 ± 6% force reduction) was statistically significant ($P < 0.05$, Student's t-test).

In the mouse, atropine was even less effective in suppressing electrically evoked contractions compared to guinea pig (see Fig. 6), in addition, higher concentrations were required for the half maximum effect than in guinea pig (Table 2). Here, too, 1 mM atropine caused some additional reduction on the muscle contraction. Propiverine and tolterodine yielded clear monophasic concentration–response curves with complete inhibition as well of the atropine-resistant component. Oxybutynin was as effective but slightly more potent ($P < 0.001$) compared with tolterodine (see Table 2).

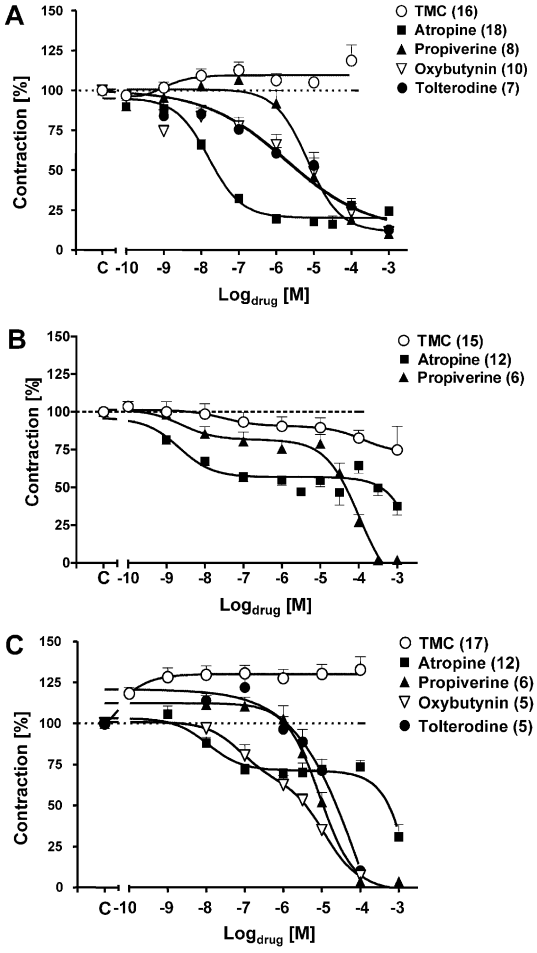

Fig. 8. Concentration-dependent effects of atropine, propiverine, oxybutynin and tolterodine on electrically stimulated force development in detrusor muscle strips from pig (A), guinea pig (B) and mouse (C) in comparison to time-matched control (TMC) recordings. The amplitude of the contraction force is expressed in percent of the pre-drug value of each individual muscle strip. Data are presented as means ± S.E.M. from n experiments. The average concentration–response curves were obtained from curve fitting to data points of each individual experiment.

Table 2
Calculated $-\log IC_{50}$ values of the investigated drugs for their effects on the electrically evoked contractions in the three different species

Species	Compound	n	$-\log IC_{50}$
Pig	atropine	18	7.82 ± 0.08
	tolterodine	7	5.95 ± 0.64
	oxybutynin	10	5.24 ± 0.32
	propiverine	8	4.73 ± 0.12
Guinea pig	atropine	12	8.77 ± 0.17
	propiverine	6	8.95 ± 0.64; 4.08 ± 0.08
Mouse	atropine	15	7.97 ± 0.09
	tolterodine	5	4.74 ± 0.16
	oxybutynin	5	5.59 ± 0.16
	propiverine	6	5.06 ± 0.07

Data in mean ± S.E.M.; IC_{50} in mol/l.

4. Discussion

In the present study, we have investigated species differences in contractile responses to carbachol and electric field stimulation of detrusor muscles of pig, guinea pig and mouse as well as differences in the suppression of these responses by atropine, propiverine, oxybutynin and tolterodine. The main findings were: (i) phasic contractions elicited by carbachol showed an order of sensitivity of guinea pig>pig>mouse. (ii) Contractile responses to electric field stimulation exhibited similar frequency dependence though they differed in amplitudes as well as in fractions of maximum contraction resistant to atropine and tetrodotoxin. (iii) The effects of propiverine, oxybutynin and tolterodine were different in the three species, underlining the importance of the animal model for the interpretation of drug action.

4.1. Carbachol concentration–response curves

Contractile responses to carbachol consisted of a rapid increase in force development that passed through a maximum and relaxed to an increasingly larger extent with higher concentrations. Compared with the literature, this general pattern was superimposed by oscillations in tension development that were more prominent in small mammals like rabbit than in pig and man (Sibley, 1984). When normalised to wet weight, the absolute values of force increase reported here are in reasonable agreement with the literature, for instance, in guinea pig detrusor muscle, 100 μM carbachol induced a maximum force development of 6.2 ± 0.9 ($n = 12$) mN/mg ww (see Section 3.1) as compared with 0.04 g/mm length × mg ww as reported by Pessina et al. (2001). Assuming an average muscle length of 8 mm, this value is equivalent to 3.2 mN/mg ww.

The reasons for the differences in sensitivity to carbachol are not clear. Species-dependent differences in muscarinic receptor densities and/or in receptor subtypes may contribute to the observed effects.

4.2. Antagonistic effects on the concentration–response curve of carbachol

The antimuscarinic effects of the four spasmolytics were studied using cumulative concentration–response curves for carbachol. The rightward shift of the curves in time-matched control experiments could be explained by a desensitization of the muscarinic receptors. In accordance with the literature, atropine was a competitive antagonist at muscarinic receptors as indicated by the rightward shift (Wada et al., 1995; Yono et al., 2000). However, propiverine, must have an additional mechanism of action at least in pig and mouse detrusor. Propiverine (10 μM) suppressed the maximum carbachol-induced contraction significantly in pig and even more so in mouse detrusor. The effect in mouse was more pronounced than previously reported in human detrusor for the maximum acetylcholine-induced contraction (Wada et al., 1995).

Interestingly, 1 μM oxybutynin shifted the curves to the right by almost three orders of magnitude in both species, however, the additional shift using 10 μM was also accompanied by a diminished maximum response in the pig but not in the mouse. With 10 μM oxybutynin, suppression of the maximum contractile response to acetylcholine has also been reported previously in the human detrusor (Wada et al., 1995).

For tolterodine, this species difference was even more pronounced, since the drug always depressed the maximum response in pig detrusor, whereas in mouse, it only shifted the concentration–response curve without affecting the maximum. Since our results in mouse but not in pig detrusor agree with previously published effects of tolterodine in human detrusor (Yono et al., 2000), we conclude that the similarity between pig and human bladder is not general but may depend on the drug investigated. One reason for the depressed maximum of the carbachol effect could be a nonspecific blocking effect on force of contraction of the detrusor muscle (Wada et al., 1995). Further experiments are required to elucidate these additional drug actions.

4.3. Contractions elicited by electric field stimulation

In all three, detrusor muscles electrically induced contractions depending on the impulse frequency during the 2-s lasting trains of stimuli. Threshold was 2 Hz, maximum contractile responses were obtained with 30–40 Hz. Since inhibitory effects of antimuscarinic agents are reported for higher frequencies (Masuda et al., 1995) the frequency of 30 Hz was chosen for the investigation of the drugs.

Electric field stimulation is considered to excite intramural nerve endings leading to release of acetylcholine and other neurotransmitters that stimulate the smooth muscle to contract (for review see Andersson, 1993). The cholinergic component of electrically elicited contractions was determined in the presence of the nonselective muscarinic receptor blocker atropine (10 μM). After blockade of neuronal excitation with 1 μM tetrodotoxin (Sibley, 1984) only direct stimulation of smooth muscle cells by electric field stimulation is observed. This myogenic component of contraction was largest in guinea pig muscle followed by pig and mouse muscle.

Time-matched control experiments were performed in order to test the stability of the preparations in the course of an experiment. Contractile responses to electric field stimulation declined in pig and guinea pig and increased in mouse substantially within the initial 30 min of regular stimulation (one train of stimuli every 2 min) and then became sufficiently stable for drug testing (see Figs. 7 and 8).

In pig, $18 \pm 2\%$ ($n = 15$) of electrically induced contractions were resistant to atropine and $11 \pm 1\%$ ($n = 15$) were resistant to tetrodotoxin, indicating that in this species—like in humans, most of the contraction is mediated by neuronal release of acetylcholine (Sibley, 1984). The respective atropine- and tetrodotoxin-resistant components of electrically induced contractions were $44 \pm 2\%$ ($n = 6$) and $24 \pm 3\%$ ($n = 6$) in guinea pig detrusor muscle. In these species, there is also evidence for contribution of Ca^{2+} influx to the large NANC component of contraction (Wada et al., 1995). The atropine-resistant component of electrically elicited contractions was largest in mouse detrusor muscle, i.e. $70 \pm 3\%$ ($n = 11$), the tetrodotoxin-resistant component was $7 \pm 1\%$ ($n = 11$). Interestingly, contraction amplitudes of mouse muscle strips significantly increased during the first 120 min in time-matched control experiments, the reason for this phenomenon is not known. Taken together, our findings indicate substantial species differences with respect to atropine- and tetrodotoxin-resistant components of electrically elicited contractions of detrusor muscles.

4.4. Concentration–response curves of the bladder spasmolytic drugs

All compounds investigated inhibited electrically induced contractions in a concentration-dependent manner. The order of potency in pig detrusor muscle was atropine> propiverine ≈ tolterodine ≈ oxybutynin and is comparable with less complete data published for human tissue, i.e. oxybutynin ≈ tolterodine (Ückert et al., 2000) or atropine>oxybutynin ≈ propiverine (Wada et al., 1995). The similarity in drug order of potency confirms previous data of similarity of contraction mechanisms in pig and human detrusor muscle.

In pig detrusor muscle, the concentration–response curves for oxybutynin and tolterodine were significantly flatter than for propiverine. Atropine was between 2 and 3

log units more potent than tolterodine, oxybutynin and propiverine, but even at 1 mM, none of the agents could completely suppress contractions suggesting that they are ineffective against the myogenic component.

In guinea pig detrusor muscle, the concentration–response curve for propiverine was clearly biphasic with about 20% of suppression of tension development in the same concentration range as atropine and the remainder being blocked with a $-\log IC_{50}$ of 4.1. It should be noted that the blocking effect was complete, indicating that the myogenic component was also affected in these species.

In mouse detrusor muscle, oxybutynin exhibited a flat concentration–response curve, and although a two-component sigmoidal function did not result in a statistically better fit, the findings may be interpreted as two distinct modes of action of the drug. Force development in mouse detrusor could have been depressed partly by an antimuscarinic, partly by an additional mode of action that could relate to the Ca^{2+} channel blocking effect of oxybutynin (Comer and Goa, 2000). Propiverine and tolterodine, on the other hand, exhibited steep concentration–response curves with complete suppression of contractile responses including the tetrodotoxin-resistant fraction.

In pig detrusor muscle, the concentration–response curve for both tolterodine and oxybutynin were flat (possibly with two components), whereas in mouse muscle, the concentration–response curves for tolterodine were steep and only the one for oxybutynin was flat. The two concentrations range in which the drugs act could represent antimuscarinic and Ca^{2+} channel effects or antimuscarinic actions mediated by different subtypes. In any case, the differences between pig and mouse detrusor muscle suggest that tolterodine has only one kind of action in mouse but both actions in pig. Since Nilvebrant et al. (1997) failed to demonstrate Ca^{2+} blocking effects for tolterodine and oxybutynin at 1000-fold higher concentrations than required for block of carbachol-induced contractions (guinea pig) or electrically elicited contractions (humans), the flat concentration–response curves could imply that different muscarinic receptor subtypes are involved in electrically induced contractions in pig and mouse, and that oxybutynin and tolterodine differ in their relative affinity towards those subtypes. Oxybutynin was in fact shown to have higher affinity for M_1- and M_3- than for M_2-receptors (Noronha-Blob and Kachur, 1991), whereas tolterodine has similar affinity to all human muscarinic receptors (Nilvebrant et al., 1997).

Our findings cannot be reconciled with the assumed pharmacological action of tolterodine and propiverine. If both were pure antimuscarinic drugs with albeit lower receptor affinity than atropine, they should not reduce the maximum carbachol-induced contractions and they should not be able to depress atropine-resistant force as they did. If this extra reduction of the electrically induced contraction in the mouse was due to impairment of Ca^{2+} influx, it should be absent with tolterodine, which is said to bear only antimuscarinic properties. Clearly, these discrepancies can only be resolved in further studies and stress the importance of identical experimental conditions and animal models for comparative drug investigations.

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