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Pharmacological effects of tolterodine on human isolated urinary bladder

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Abstract

Tolterodine, (*R*)-*N*,*N*-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine, is an antimuscarinic drug developed for the treatment of overactive bladder with symptoms of frequency, urgency and urge incontinence. We investigated the effects of tolterodine and its major active metabolite, DD 01 (PNU-200577), (*R*)-*N*,*N*-diisopropyl-3-(2-hydroxy-5-hydroxymethylphenyl)-3-phenylpropanamine, on the contractions induced by carbachol, KCl, $CaCl_2$ and electrical field stimulation in human isolated urinary bladder smooth muscles, using the muscle bath technique. Specimens of human urinary bladder were obtained from 20 patients who underwent total cystectomy due to malignant bladder tumor. The detrusor preparations were taken from the intact part of the dome region of the bladder. Carbachol (10^{-9}–10^{-2} M) caused concentration-dependent contraction of human detrusor smooth muscles. Tolterodine (10^{-9}–10^{-6} M), DD 01 (10^{-9}–10^{-6} M), oxybutynin (10^{-8}–10^{-6} M), propiverine (10^{-8}–10^{-6} M), atropine (10^{-9}–10^{-6} M), pirenzepine (10^{-8}–10^{-5} M), methoctramine (10^{-8}–10^{-5} M) and 4-diphenylacetoxy-*N*-methylpiperidine (4-DAMP) (10^{-9}–10^{-6} M) caused typical shifts to the right of the concentration–response curves for carbachol, except for higher concentrations (10^{-5} M) of oxybutynin and propiverine, which caused a decrease of about 30% of the maximum contractile responses to carbachol. All the slopes of the regression lines of Schild plots were close to unity, and the rank order of pA_2 values was: atropine = DD 01 = tolterodine = 4-DAMP = oxybutynin > propiverine = pirenzepine > methoctramine. Tolterodine (10^{-9}–10^{-6} M) and DD 01 (10^{-9}–10^{-6} M) did not inhibit the KCl-induced (80 mM) and $CaCl_2$-induced (5 mM) contractions, while oxybutynin (10^{-8}–10^{-5} M) and propiverine (10^{-8}–10^{-5} M) significantly inhibited the contractions. Electrical field stimulation (2–60 Hz) caused frequency-dependent contraction of human detrusor smooth muscles, which were significantly inhibited by various drugs. In the presence of 10^{-6} M atropine, tolterodine and DD 01 did not inhibit the residual contractions induced by electrical field stimulation at any of the frequencies, while oxybutynin (10^{-5} M) and propiverine (10^{-5} M) significantly inhibited the atropine-resistant part of the contractions. The results suggest that the inhibitory effects of tolterodine and DD 01 are mediated only by their antimuscarinic action, which is equal to that of oxybutynin and significantly greater than that of propiverine, and that tolterodine and DD 01 have neither Ca^{2+} channel antagonist action nor inhibitory effect on the atropine-resistant part of the contractions in human detrusor smooth muscles. These findings support the usefulness of tolterodine as a therapeutic drug for overactive bladder with symptoms of frequency, urgency and urge incontinence.

Keywords: Tolterodine; DD 01; Antimuscarinic drug; Urinary bladder; (Human)

1. Introduction

Parasympathetic nerve stimulation initiates contraction of the human urinary bladder (Steers, 1997). A major portion of the neurohumoral stimulus for physiological bladder contraction is the acetylcholine-induced stimulation of postganglionic parasympathetic muscarinic receptors on the bladder smooth muscle. It has been reported that atropine and atropine-like agents (antimuscarinic drugs) depress uninhibited contractions (Blaivas et al., 1980; Jensen, 1981) and bring about a significant clinical improvement in patients with detrusor instability and hyperreflexia.

Tolterodine, (*R*)-*N*,*N*-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine, is an antimuscarinic drug developed for the treatment of overactive bladder with symptoms of frequency, urgency and urge incontinence. Pharmacological studies (Nilvebrant et al., 1994, 1997a,c) have demonstrated that this compound has no Ca^{2+} channel antagonist action. In addition, tolterodine has been shown to exhibit favorable selectivity for the bladder as compared with the salivary glands, in vivo, while oxybutynin shows the opposite selectivity (Nilvebrant

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et al., 1994, 1997a,c). The potent antimuscarinic action had been demonstrated on the human urinary bladder (Naerger et al., 1995; Nilvebrant et al., 1995). In the human, the major metabolic pathway of tolterodine involves hydroxylation of the 5-methyl group, resulting in the 5-hydroxymethyl derivative DD 01 (PNU-200577), (*R*)-*N*,*N*-diisopropyl-3-(2-hydroxy-5-hydroxymethylphenyl)-3-phenylpropanamine, which is a major pharmacologically active metabolite (Nilvebrant et al., 1997b,c). However, little information is as yet available on the effects of these compounds on the human urinary bladder in vitro. We therefore attempted to evaluate the effects of tolterodine and the active metabolite DD 01 in comparison with those of a series of reference compounds, using the muscle bath technique.

2. Materials and methods

2.1. Tissues

Specimens of human urinary bladder were obtained from 20 patients (18 males and two females, 67.4 ± 1.7 years old) who underwent total cystectomy due to malignant bladder tumor. We obtained permission for using human bladder tissue from the Ethics Committee at Kumamoto University School of Medicine. We also obtained informed consent from the patient or patient's family before surgery. All patients did not receive preoperative local radiotherapy and/or chemotherapy.

After surgical removal of the bladder, the tissue was immediately immersed in modified Krebs–Henseleit solution of the following composition (in mM): NaCl 117.70, KCl 4.69, $CaCl_2$ 2.16, $MgSO_4$ 1.20, $NaHCO_3$ 24.39, KH_2PO_4 1.20 and glucose 9.99; 37°C at pH 7.4, gassed with 95% O_2–5% CO_2. The serosal and mucosal layers were dissected and detrusor strips were cut (approximately 4 mm wide and 15 mm long) from the intact part of the dome region of the bladder.

2.2. Functional experiments

The functional experiments were performed as previously described (Yoshida et al., 1992; Wada et al., 1995). The detrusor strip was suspended in a 20-ml bath filled with modified Krebs–Henseleit solution. Each muscle preparation was connected to a force displacement transducer (TB-611T; Nihon Kohden, Tokyo, Japan) and isometric forces were recorded and monitored on an ink-writing recorder (R-02A; Rika Denki, Tokyo, Japan). After each strip was stretched until optimal force developed (about 1.5 g resting tension), the strip was washed with modified Krebs–Henseleit solution several times and allowed to equilibrate for 90 min before the start of the experiments.

Concentration–response curves for carbachol were obtained in a stepwise manner after the response to the previous concentration had reached a plateau. After cumulative concentration–response curves were made in the absence of any antagonist, the strips were washed with modified Krebs–Henseleit solution several times and allowed to relax to baseline. After 60 min, the strips were incubated with tolterodine, DD 01, oxybutynin, propiverine, atropine, pirenzepine, methoctramine or 4-diphenylacetoxy-*N*-methylpiperidine (4-DAMP) for 30–60 min and the concentration–response curves for carbachol were then obtained in the presence of increasing concentrations of each antagonist.

To assess the potency of the antimuscarinic action, the dose ratio was obtained from the ratio of the ED_{50} values (the concentration of an agonist producing 50% of the maximum contraction) for the carbachol-induced contractions in the presence and in the absence of antagonists. The Schild plots were obtained by plotting the log (dose ratio —1) against the log molar concentration of the antagonist, and the pA_2 values were derived from the Schild plots (Arunlakshana and Schild, 1959).

In the studies on KCl-induced contractions, control contractile responses to 80 mM of KCl were obtained by equimolar replacement of NaCl by KCl in modified Krebs–Henseleit solution, which was gassed and maintained at 37°C. The strips were then washed with modified Krebs–Henseleit solution several times. For $CaCl_2$-induced contractions, Ca^{2+} was eliminated from the modified Krebs–Henseleit solution and 0.1 mM EGTA was added to chelate the trace amount of Ca^{2+}. The strips were incubated in this medium for 20 min (washed every 5 min with Ca^{2+}-free medium), before control contractile responses to 5 mM of $CaCl_2$ were obtained by adding $CaCl_2$. The strips were then washed with Ca^{2+}-free medium every 5 min for 20 min. In both experiments, the strips were incubated with tolterodine, DD 01, oxybutynin or propiverine for 30–60 min, and KCl- or $CaCl_2$-induced contractions were measured in the presence of increasing concentrations of each drug.

Table 1
E_{max} value for carbachol-, KCl (80 mM)-, $CaCl_2$ (5 mM)- and electrical field stimulation (0.3-ms duration, 3-s train, 2-min interval) -induced contractions of human urinary bladder smooth muscles

	E_{max} (g)
Carbachol	4.83 ± 0.17[a]
KCl	3.57 ± 0.23
$CaCl_2$	2.93 ± 0.15
Electrical field stimulation	4.54 ± 0.31[b]

Values for carbachol, KCl, $CaCl_2$ and electrical field stimulation represent means ± S.E.M. of the results from 32, 16, 16 and 40 separate experiments, respectively.
[a] Significantly different from comparable values for KCl ($P < 0.005$) and $CaCl_2$ ($P < 0.0001$).
[b] Significantly different from comparable values for KCl ($P < 0.05$) and $CaCl_2$ ($P < 0.0005$).

For electrical field stimulation, two platinum wire electrodes (10 mm wide and 8 mm apart) were set parallel to each other one on either side of the strips. Electrical impulses for field stimulation of the intramural nervous system were delivered with a stimulator (SEN-3301; Nihon Kohden) and boosted by an amplifier (SEG-3104;

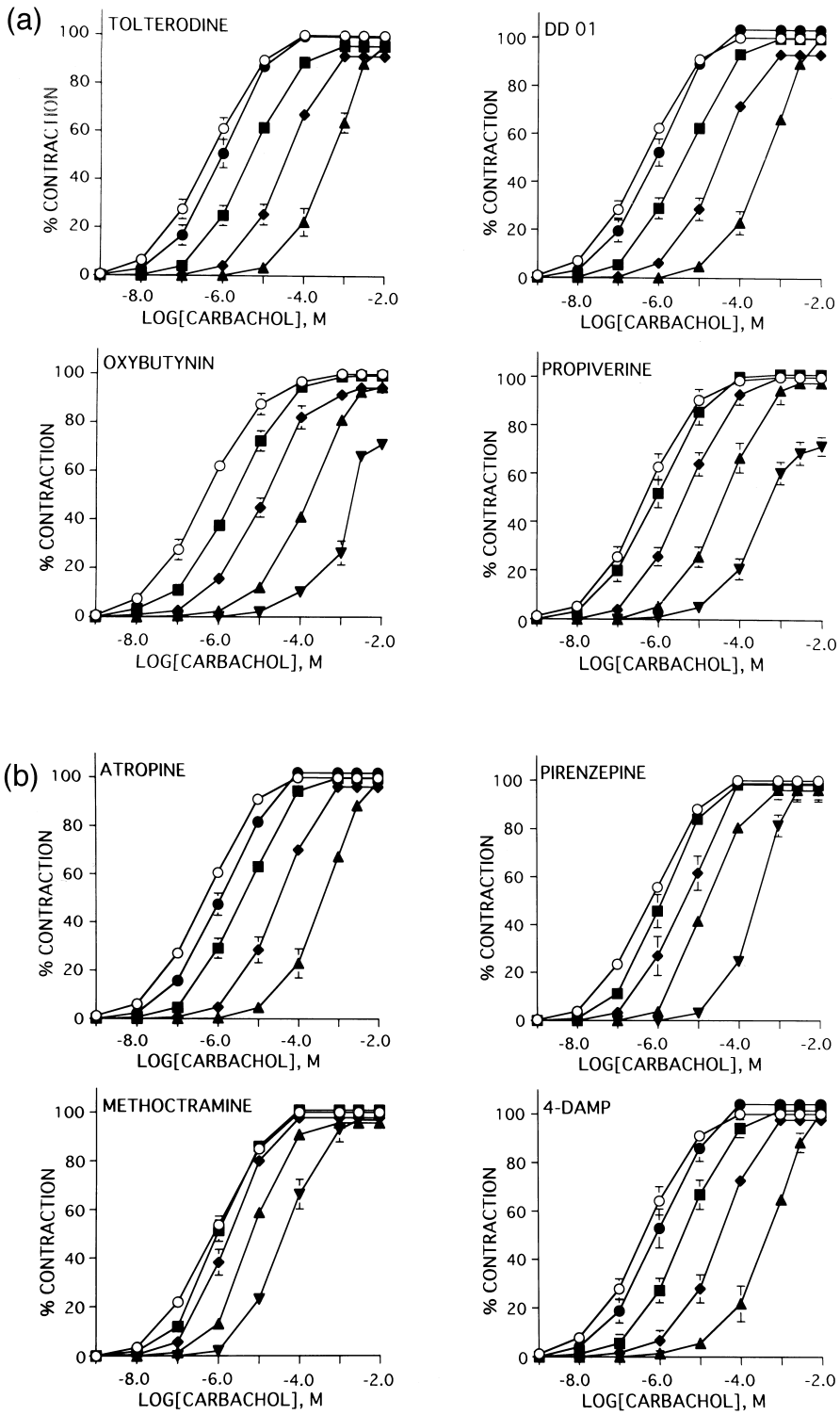

Fig. 1. Effects of various antimuscarinic drugs ((a) tolterodine, DD 01, oxybutynin and propiverine, (b) selective muscarinic receptor antagonists) on the concentration–response curves for carbachol in human urinary bladder smooth muscles. ○ Control, ● 10^{-9} M, ■ 10^{-8} M, ◆ 10^{-7} M, ▲ 10^{-6} M, ▼ 10^{-5} M. For each experiment, contractile responses are expressed as percentages of the maximum contractile response in the absence of any antagonist. Each point represents the mean ± S.E.M. of the results from four separate experiments; if not shown, S.E. bars fall within the size of the symbols.

Table 2
pA_2 values and slopes of Schild plots for various drugs on human urinary bladder smooth muscles

Drugs	pA_2	Slope
Tolterodine	9.04 ± 0.10	0.97 ± 0.04
DD 01	9.04 ± 0.13	0.97 ± 0.05
Oxybutynin	8.63 ± 0.11	0.97 ± 0.04
Propiverine	7.94 ± 0.06	1.01 ± 0.05
Atropine (non-selective)	9.06 ± 0.09	0.93 ± 0.04
Pirenzepine (M_1 selective)	7.82 ± 0.31	0.94 ± 0.18
Methoctramine (M_2 selective)	7.14 ± 0.35	0.90 ± 0.18
4-DAMP (M_3 selective)	8.93 ± 0.19	1.03 ± 0.11

Values represent means ± S.E.M. of the results from four separate experiments.

Nihon Kohden). The intrinsic nerves were stimulated with rectangular pulses of 0.3-ms duration and supramaximal voltage, at stimulation frequencies of 2 to 60 Hz. Trains of pulses lasted for 3 s and an interval of 2 min between stimulations was used. After the frequency–response curves in the absence of antagonists were obtained, the strips were incubated with each drug for 30–60 min, before the frequency–response curves were made in the presence of increasing concentrations of each drug. In preliminary experiments, atropine (10^{-9}–10^{-5} M) was used to establish that the maximum inhibition of the electrical field stimulation-induced contractions was obtained at 10^{-6} M. Therefore, 10^{-6} M atropine was routinely used in the main experiments. To evaluate the effects of tolterodine, DD 01, oxybutynin or propiverine on the atropine-resistant part of the contractions, frequency–response curves were made in the presence of 10^{-6} M atropine and increasing concentrations of each drug.

Before and after the experiments, the KCl-induced (80 mM) contractions were measured in each preparation to check contractility. The contractile responses to KCl were not significantly altered during the course of the experiments. In preliminary experiments, the concentration–response curve to carbachol, or the contractile responses to KCl (80 mM) or $CaCl_2$ (5 mM) without any antagonist, were made every 1 h with the same strip. The contractile responses were stable for 9 to 10 h.

2.3. Drugs

The following pharmacological agents were used: atropine sulfate, carbamylcholine chloride (carbachol), oxybutynin chloride and tetrodotoxin (Sigma, Tokyo, Japan); pirenzepine dihydrochloride, methoctramine tetrahydrochloride and 4-DAMP methiodide (Funakoshi, Tokyo, Japan). Tolterodine, DD 01 and propiverine were kindly donated by Pharmacia and Upjohn, Sweden. Other chemicals and materials were of analytical grade and obtained from commercial sources. Concentrations are expressed as final bath concentrations. Drugs were dissolved in distilled water and volumes of 0.2 ml were added to the bath.

2.4. Data analyses

The E_{max} value (the maximum contractile response) was obtained from the maximum stress developed, and the ED_{50} value was calculated from a semilogarithmic plot of the percentage of the maximum response vs. drug concentration. Statistical analyses for comparisons between groups and between concentration–response curves were performed using the analysis of variance (ANOVA) and the multiple comparison test (Fisher's test).

3. Results

3.1. Effects of various antimuscarinic drugs on carbachol-induced contractions

Carbachol (10^{-9}–10^{-2} M) caused concentration-dependent contraction of human detrusor smooth muscles. The E_{max} and ED_{50} values for the carbachol-induced contractions were 4.83 ± 0.17 g (Table 1) and 0.56 ± 0.05 μM. Tolterodine (10^{-9}–10^{-6} M), DD 01 (10^{-9}–10^{-6} M), oxybutynin (10^{-8}–10^{-6} M), propiverine (10^{-8}–10^{-6} M), atropine (10^{-9}–10^{-6} M), pirenzepine (10^{-8}–10^{-5} M), methoctramine (10^{-8}–10^{-5} M) and 4-DAMP (10^{-9}–10^{-6} M) caused typical shifts to the right of the concentra-

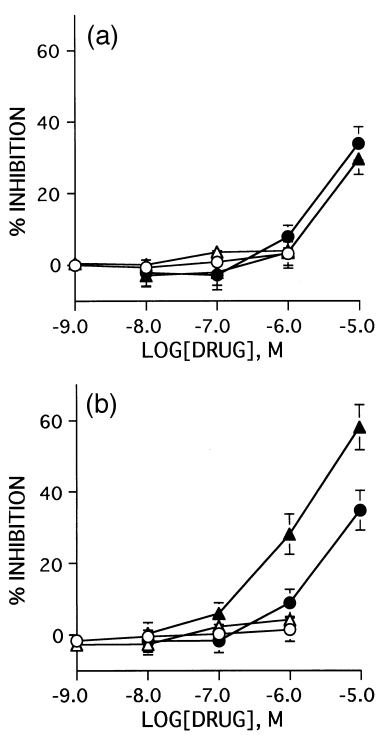

Fig. 2. Effects of various antimuscarinic drugs on (a) KCl-induced (80 mM) and (b) $CaCl_2$-induced (5 mM) contractions in human urinary bladder smooth muscles. ○ Tolterodine, △ DD 01, ● oxybutynin, ▲ propiverine. Each point represents the mean ± S.E.M. of the results from four separate experiments; if not shown, S.E. bars fall within the size of the symbols.

tion–response curves for carbachol, except for higher concentrations (10^{-5} M) of oxybutynin and propiverine, which also caused a decrease of about 30% of the maximum contractile responses to carbachol (Fig. 1a,b).

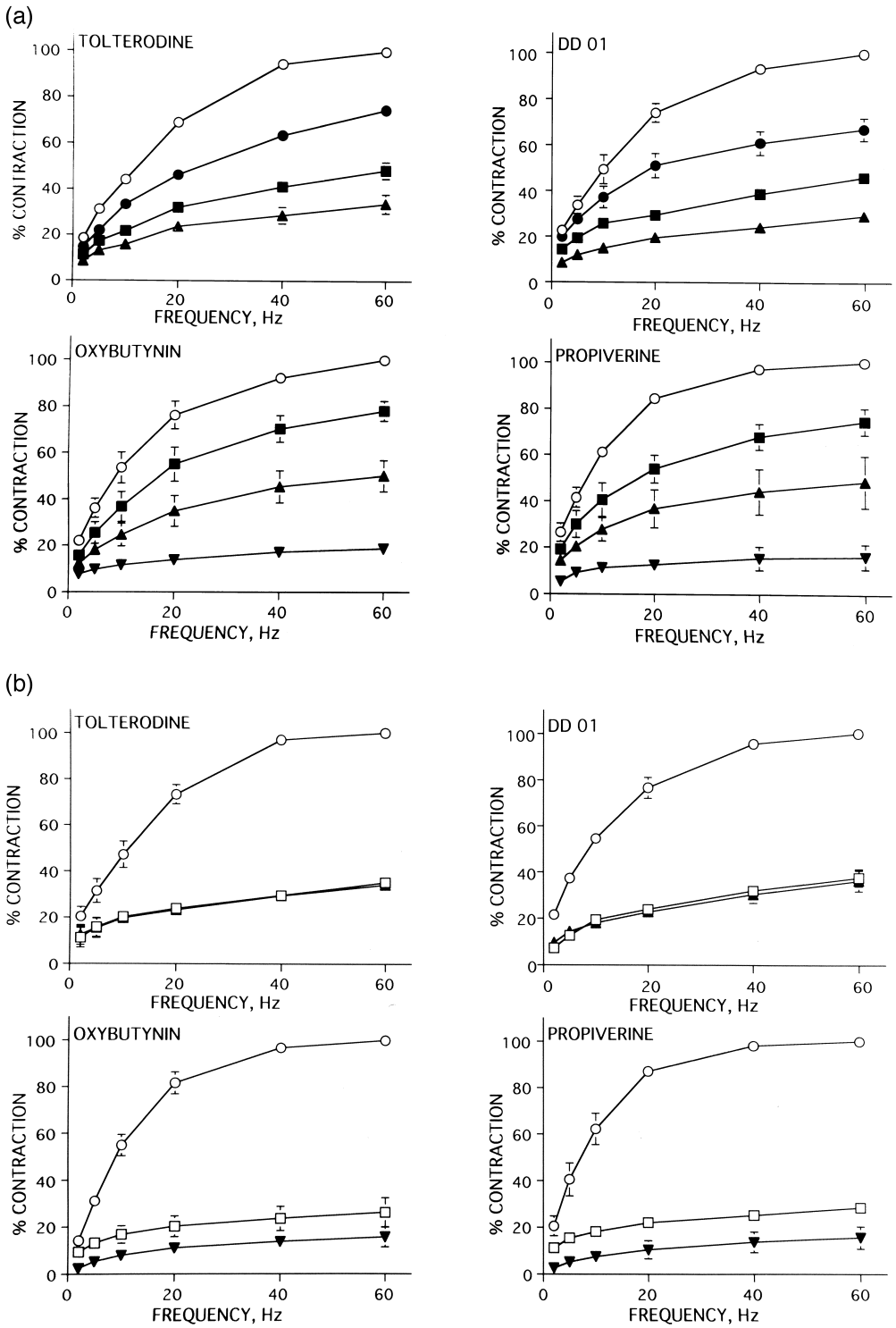

Fig. 3. Effects of various antimuscarinic drugs on the frequency–response curves for electrical field stimulation (0.3-ms duration, 3-s train, 2-min interval) in human urinary bladder smooth muscles ((a) in the absence of atropine, (b) in the presence of 10^{-6} M atropine). ○ Control, ● 10^{-8} M, ■ 10^{-7} M, ▲ 10^{-6} M, ▼ 10^{-5} M, □ 10^{-6} M atropine. For each experiment, contractile responses at given frequencies are expressed as percentages of the contractile response at 60 Hz in the absence of any drug. Each point represents the mean ± S.E.M. of the results from four to six separate experiments; if not shown, S.E. bars fall within the size of the symbols.

The pA_2 values for tolterodine, DD 01, oxybutynin, propiverine, atropine, pirenzepine, methoctramine and 4-DAMP were calculated from the concentration–response curves at the concentrations described above. The pA_2 values and slopes of the Schild plots for the various drugs tested, are shown in Table 2. All the slopes of the regression lines were close to unity, indicating that each drug competitively antagonized the contractile response. The rank order of pA_2 values was: atropine = DD 01 = tolterodine = 4 - DAMP = oxybutynin > propiverine = pirenzepine > methroctamine.

3.2. Effects of various antimuscarinic drugs on KCl- and $CaCl_2$-induced contractions

The E_{max} values for the 80 mM KCl- and 5 mM $CaCl_2$-induced contractions were 3.57 ± 0.23 and 2.93 ± 0.15 g, respectively (Table 1). Tolterodine (10^{-9}–10^{-6} M) and DD 01 (10^{-9}–10^{-6} M) did not inhibit the KCl- and $CaCl_2$-induced contractions, while oxybutynin (10^{-8}–10^{-5} M) and propiverine (10^{-8}–10^{-5} M) concentration dependently inhibited the responses (Fig. 2a,b). The inhibition of oxybutynin (10^{-5} M) for KCl- and $CaCl_2$-induced contractions was $33.9 \pm 4.7\%$ and $34.9 \pm 5.5\%$, respectively. Propiverine (10^{-5} M) also inhibited the contractions by $29.5 \pm 4.3\%$ and $58.2 \pm 6.3\%$, respectively.

3.3. Effects of various antimuscarinic drugs on contractions induced by electrical field stimulation

Electrical field stimulation (2–60 Hz) caused frequency-dependent contraction of human detrusor smooth muscles. The E_{max} value was 4.54 ± 0.31 g (Table 1). The contractions induced by electrical field stimulation were completely suppressed by pretreatment with 10^{-6} M of tetrodotoxin. Tolterodine (10^{-8}–10^{-6} M), DD 01 (10^{-8}–10^{-6} M), oxybutynin (10^{-7}–10^{-5} M) and propiverine (10^{-7}–10^{-5} M) significantly inhibited the frequency–response curves for electrical field stimulation (Fig. 3a). The maximum inhibition by tolterodine, DD 01, oxybutynin, propiverine and atropine was $52.9 \pm 10.2\%$, $57.9 \pm 7.1\%$, $65.6 \pm 4.7\%$, $79.7 \pm 3.8\%$ and $48.9 \pm 4.7\%$ at 2 Hz, and $66.2 \pm 4.1\%$, $70.8 \pm 1.0\%$, $81.4 \pm 2.5\%$, $83.8 \pm 5.4\%$ and $67.2 \pm 2.1\%$ at 60 Hz, respectively. In the presence of 10^{-6} M atropine, tolterodine (10^{-6} M) and DD 01 (10^{-6} M) did not inhibit the atropine-resistant part of the contractions induced by electrical field stimulation at any of the frequencies, while oxybutynin (10^{-5} M) and propiverine (10^{-5} M) significantly inhibited the atropine-resistant part of the contractions (Fig. 3b).

4. Discussion

In the present study, tolterodine (10^{-9}–10^{-6} M), DD 01 (10^{-9}–10^{-6} M), oxybutynin (10^{-8}–10^{-6} M), propiverine (10^{-8}–10^{-6} M), atropine (10^{-9}–10^{-6} M), pirenzepine (10^{-8}–10^{-5} M), methoctramine (10^{-8}–10^{-5} M) and 4-DAMP (10^{-9}–10^{-6} M) caused parallel shifts to the right of the concentration–response curves for carbachol, without significant decreases in maximum contractile responses. Furthermore, all the slopes of the regression lines of Schild plots were close to unity. These findings suggest that all drugs competitively antagonized the contractile responses at the concentrations described above. Based on the pA_2 values, the rank order of antimuscarinic action was: atropine = DD 01 = tolterodine = 4-DAMP = oxybutynin > propiverine = pirenzepine > methoctramine.

Recently, five pharmacological subtypes of muscarinic receptors have been found, from M_1 to M_5 (Caulfield and Birdsall, 1998), and five distinct genes encoding muscarinic receptors have been cloned and designated as M_1–M_5 genes (Dörje et al., 1991; Caulfield and Birdsall, 1998). The human urinary bladder smooth muscles contain a mixed population of the muscarinic M_2 and M_3 receptors, in common with those of other mammalian species. Furthermore, it has been demonstrated that the muscarinic M_2 receptors predominate, but the contractile response is mediated by the muscarinic M_3 receptors (Kondo and Morita, 1993; Wang et al., 1995; Mutoh et al., 1997). In the present study, we investigated the effects of pirenzepine (muscarinic M_1 receptor selective), methoctramine (muscarinic M_2 receptor selective), 4-DAMP (muscarinic M_3 receptor selective) and atropine (non-selective) on carbachol-induced contractions. The pA_2 value of 8.93 for 4-DAMP was within the range of 8.6–9.2 obtained for the muscarinic M_3 receptors in various smooth muscle preparations (Batink et al., 1987; Eglen et al., 1987; Gater et al., 1987; Moore et al., 1988; Roffel et al., 1988). Similarly, the pA_2 value of 7.14 for methoctramine was within the range of 6.9–7.5 obtained for the muscarinic M_2 receptors in the heart (Batink et al., 1987; Duckles et al., 1987; Micheletti et al., 1987). However, the pA_2 value of 7.82 for pirenzepine found in the present study was smaller than the values obtained for the muscarinic M_1 receptors both in rat cerebral cortex (8.4) (Smith and Yamamura, 1985) and in rabbit vas deferens (8.2) (Lambrecht et al., 1989). In addition, the postjunctional muscarinic M_1 receptors have never been demonstrated in the human urinary bladder smooth muscles (Yamaguchi et al., 1994; Wang et al., 1995). Thus, these results suggest that the contractile response may be mediated not only by the muscarinic M_3 receptors, but also by the muscarinic M_2 receptors in the human urinary bladder smooth muscles. In agreement with this, Gillberg et al. (1998) have shown that the muscarinic M_2 receptors may have an important role in bladder contraction.

Radioligand binding studies showed tolterodine and DD 01 to have no subtype selectivity (Nilvebrant et al., 1997a,b,c). These findings may imply that blockade of not only the muscarinic M_3 receptors but also the muscarinic M_1 and/or M_2 receptors in the bladder contributes to the tissue selectivity of tolterodine and DD 01 demonstrated in vivo. It has been reported that the prejunctional muscarinic

M_1 receptors have facilitating effects on acetylcholine release (Somogyi et al., 1994; Tobin and Sjögren, 1995), and that the postjunctional muscarinic M_2 receptors inhibit β-adrenoceptor-stimulated adenylate cyclase activity in modulating smooth muscle relaxation (Eglen et al., 1994). Thus, the blockade of the prejunctional muscarinic M_1 and the postjunctional muscarinic M_2 receptors by tolterodine and DD 01 may contribute to the inhibitory effects of these drugs on detrusor contractions. In addition, animal studies showed tolterodine and DD 01 to exhibit selectivity for the bladder over salivary glands in vivo (Nilvebrant et al., 1997a,b,c). Several recent reports suggest that salivary glands contain almost exclusively muscarinic M_3 receptors, and that selectivity for the muscarinic M_3 over the muscarinic M_2 receptors in vitro, which was observed for oxybutynin (Nilvebrant et al., 1997a,c), may result in a more pronounced effect on salivation than on bladder contraction in vivo (Nilvebrant et al., 1997a; Gillberg et al., 1998).

The present study showed that the maximum contractile responses to carbachol were significantly inhibited by high concentrations (10^{-5} M) of oxybutynin and propiverine, and that the inhibition of contractions induced by KCl (80 mM) and $CaCl_2$ (5 mM) was also seen with as little as 10^{-6} M of oxybutynin and propiverine on human detrusor muscles. The data are consistent with previous reports that oxybutynin and propiverine have significant Ca^{2+} channel antagonist actions (Aida et al., 1986; Haruno et al., 1989; Wada et al., 1995). In the human urinary bladder, the IC_{50} values for oxybutynin and propiverine were 12.4 and 14.5 μM for KCl-induced contractions, and 8.7 and 2.2 μM for $CaCl_2$-induced contractions, respectively (Wada et al., 1995). However, the Ca^{2+} channel antagonist actions of oxybutynin and propiverine may not be relevant in the clinical situation, since Ca^{2+} channel antagonist actions occur at very high concentrations compared with the antimuscarinic actions of these drugs. On the other hand, the effects of tolterodine and DD 01 on KCl- and $CaCl_2$-induced contractions were negligible, which is consistent with previous reports (Nilvebrant et al., 1994, 1997a,c). These results suggest that the efficacy of the Ca^{2+} channel antagonist action differs among the 4 drugs used in our study, and that, at the concentrations tested, tolterodine and DD 01 have no Ca^{2+} channel antagonist action in human detrusor smooth muscles.

In the present study, 10^{-6} M of tetrodotoxin completely suppressed the electrical field stimulation-induced contraction of the human detrusor smooth muscle, suggesting that this kind of stimulation evokes pure neurogenic contractions. All drugs had concentration-dependent inhibitory effects on the electrical field stimulation-induced contractions. The maximum inhibition differed among drugs. Oxybutynin and propiverine produced a significant inhibition of the atropine-resistant part of the contractions, which is consistent with the data from the previous report (Wada et al., 1995), while tolterodine and DD 01 had no effect on the atropine-resistant response. The exact mechanism of the atropine-resistant response to electrical field stimulation has not been clearly elucidated. However, it has been reported that a major portion of the atropine-resistant response is mediated by non-adrenergic, non-cholinergic neurotransmitters, including adenosine triphosphate (ATP), prostaglandins and vasoactive intestinal polypeptide (Luheshi and Zar, 1990). Zar et al. (1990) have shown that the atropine-resistant component of the response to nerve stimulation is more sensitive to Ca^{2+} influx than is the cholinergic component. In the present study, tolterodine and DD 01 showed no Ca^{2+} channel antagonist action. This implies that tolterodine and DD 01 cannot significantly inhibit the atropine-resistant part of the contractions, while the Ca^{2+} channel antagonist actions of oxybutynin and propiverine may be related to the inhibitory effects on the atropine-resistant part of the contractions. However, Nilvebrant et al. (1994) reported that much higher concentrations of tolterodine showed the Ca^{2+} channel antagonist activity in the guinea pig bladder, and that the IC_{50} value (6.5 μM) of tolterodine for Ca^{2+} channel antagonist action was 1100 times higher than the K_B and K_i values for muscarinic receptors. Thus, it is suggested that the effects of higher concentrations of tolterodine and DD 01 are of no clinical relevance.

In conclusion, the present study demonstrated that tolterodine and DD 01 inhibited contractions of human detrusor smooth muscles by their antimuscarinic action, which was equal to that of oxybutynin and significantly greater than that of propiverine. Tolterodine and DD 01 did not have any Ca^{2+} channel antagonist action, while oxybutynin and propiverine had both antimuscarinic and Ca^{2+} channel antagonist actions. There was, however, a large difference between the concentration ranges where the antimuscarinic and Ca^{2+} channel antagonist actions occur. These findings support the usefulness of tolterodine as a therapeutic drug for overactive bladder with symptoms of frequency, urgency and urge incontinence.

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