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TOPIC PAPER

The muscarinic receptor antagonist propiverine exhibits α_1-adrenoceptor antagonism in human prostate and porcine trigonum

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Abstract
Purpose Combination therapy of male lower urinary tract symptoms with α_1-adrenoceptor and muscarinic receptor antagonists attracts increasing interest. Propiverine is a muscarinic receptor antagonist possessing additional properties, i.e., block of L-type Ca^{2+} channels. Here, we have investigated whether propiverine and its metabolites can additionally antagonize α_1-adrenoceptors.
Methods Human prostate and porcine trigone muscle strips were used to explore inhibition of α_1-adrenoceptor-mediated contractile responses. Chinese hamster ovary (CHO) cells expressing cloned human α_1-adrenoceptors were used to determine direct interactions with the receptor in radioligand binding and intracellular Ca^{2+} elevation assays.
Results Propiverine concentration-dependently reversed contraction of human prostate pre-contracted with 10 μM phenylephrine ($-\log IC_{50}$ [M] 4.43 ± 0.08). Similar inhibition was observed in porcine trigone ($-\log IC_{50}$ 5.01 ± 0.05), and in additional experiments consisted mainly of reduced maximum phenylephrine responses. At concentrations ≥1 μM, the propiverine metabolite M-14 also relaxed phenylephrine pre-contracted trigone strips, whereas metabolites M-5 and M-6 were ineffective. In radioligand binding experiments, propiverine and M-14 exhibited similar affinity for the three α_1-adrenoceptor subtypes with $-\log K_i$ [M] values ranging from 4.72 to 4.94, whereas the M-5 and M-6 did not affect [^{3}H]-prazosin binding. In CHO cells, propiverine inhibited α_1-adrenoceptor-mediated Ca^{2+} elevations with similar potency as radioligand binding, again mainly by reducing maximum responses.
Conclusions In contrast to other muscarinic receptor antagonists, propiverine exerts additional L-type Ca^{2+}-channel blocking and α_1-adrenoceptor antagonist effects. It remains to be determined clinically, how these additional properties contribute to the clinical effects of propiverine, particularly in male voiding dysfunction.

Keywords α_1-Adrenoceptor · Bladder trigone · Propiverine · Prostate

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Introduction

The medical treatment of male lower urinary tract symptoms (LUTS) typically consists primarily of α_1-adrenoceptor antagonists, but in many patients this provides

insufficient symptom relief. A combination of α_1-adrenoceptor and muscarinic receptor antagonists may be more effective, particularly against storage symptoms [1, 2]. While most combination studies have used tolterodine as the muscarinic antagonist, several have also been based on propiverine [1–4].

Propiverine is an antagonist with similar affinity for all muscarinic receptor subtypes [5, 6] which also inhibits voltage-gated L-type Ca^{2+}-channels [9–13]. As contractions of prostate [7] and bladder smooth muscle [8] at least partly depend on such channels, their inhibition may contribute to the therapeutic effects of propiverine in LUTS suggestive of benign prostatic hyperplasia and overactive bladder (OAB), respectively.

Based upon the growing interest in a combination treatment of male LUTS, we have explored possible α_1-adrenoceptor antagonist effects of propiverine and its metabolites M-5, M-6, and M-14, which share the anti-muscarinic and/or L-type Ca^{2+}-channel-blocking activity of propiverine [16, 17].

Materials and methods

Prostate and trigone detrusor contraction

Human prostate was obtained with informed written consent in accordance with the regulations of the local hospital ethical committee (permit EK 194092004) from six patients undergoing combined prostectomy and radical cystectomy for invasive bladder cancer (65 ± 3 years). Urinary bladder trigone from juvenile and adult female pigs were obtained from a local abattoir. Prostate strips (10 mm long and 3–4 mm wide) and trigone strips (7–10 mm long and 2–4 mm wide without urothelium) were prepared and mounted in organ baths as previously described [7, 18] except for a resting load of 5 mN. After an equilibration period of 60 min, the strips were challenged with a single concentration of α_1-adrenoceptor agonist phenylephrine (10 μM) to reach a stable pre-contraction after 60 min. Then, increasing concentrations of test compounds were added cumulatively (15 min between concentration steps). Relaxation values were normalized to the 10 μM forskolin effect, as determined at the end of each experiment, and were corrected for spontaneous decline of force in time-matched control (TMC) experiments.

In other experiments, porcine trigone strips were exposed to cumulatively increasing concentrations of phenylephrine (30 nM–100 μM) with 5 min of stabilization between two subsequent additions. After maximum contraction was observed, phenylephrine was removed by washing 3 times with drug-free solution and re-equilibrating for 60 min. Then a single concentration of test compound was added and after additional 30 min, a second concentration-response curve for phenylephrine was generated.

Radioligand binding

Binding of propiverine and its metabolites to human α_1-adrenoceptor subtypes was analyzed in Chinese hamster ovary (CHO) cells, expressing approximately 2 pmol receptor/mg protein, by competition binding against [^{3}H]-prazosin as previously described [19, 20]. Phentolamine was used as a reference antagonist. Radioactivity adherent to the filters was quantified in a Topcount NXT (Perkin Elmer, Zaventem, Belgium) using Microsint O (Perkin Elmer) scintillator.

Intracellular Ca^{2+}

Cells were plated in black, clear bottom 96 wells plates at 50,000 cells per well. After 24 h in serum-free medium, cells were loaded for 1 h with 4 μM Fluo-4 AM ester in buffer (HBSS containing 20 mM HEPES and 250 mM probenecid and 0.42% v/v pluronic acid). They were then washed twice and incubated for 45 min with buffer. Fluorescence was measured using an excitation filter at 485 nm and emission filter at 520 nm on a NOVOstar (BMG Labtech, via Isogen, IJselstein, the Netherlands). After measuring the basal level for 10 s, phenylephrine (100 pM–10 μM) was added and measured for 50 s, then 5% v/v triton X-100 in basic buffer was added at 10% v/v to determine the maximal signal (F_{max}). After 20 s, 0.1 M EGTA in buffer was added at 10% v/v to determine the minimal signal (F_{min}). The increase in free intracellular Ca^{2+} ($[Ca^{2+}]_i$) was calculated as the difference between the $[Ca^{2+}]_i$ for the basal level and after adding a ligand. $[Ca^{2+}]_i$ was calculated by the equation:

$$\left[Ca^{2+}\right]_i = K_d * ((F - F_{min})/(F_{max} - F))$$

K_d is the dissociation constant of the binding of Fluo-4 to Ca^{2+} (345 nM). Concentration-response curves for phenylephrine were generated in duplicate in the absence and presence of propiverine, its metabolites M-5, M-6, and M-14 and the reference antagonist phentolamine (added 15 min prior to phenylephrine).

Data analysis

Experimental data were analyzed, by non-linear curve fitting of each individual experiment using GraphPad Prism® 4.00 (GraphPad Software, San Diego, CA, USA). Potencies of propiverine, tamsulosin, and prazosin on phenylephrine pre-contracted human prostate and porcine trigone strips

were determined as $-\log IC_{50}$ [M] values. The potency ($-\log EC_{50}$ [M]) and efficacy of phenylephrine-induced contractions and $[Ca^{2+}]_i$ elevations were determined in the absence and presence of the indicated test compounds. Maximum contraction during the second concentration-response curve for phenylephrine (Eff_{max}) was expressed as percent of the maximum effects during the first concentration-response curve (=100%). Inhibitory potency as determined from competition binding experiments was transformed to $-\log K_i$ values using the Cheng and Prusoff equation. Statistical differences were tested by Student's *t*-test and were considered significant for $P < 0.05$.

Chemicals

[^{3}H]-prazosin (specific activity 80 Ci/mmol) was purchased from Perkin Elmer (Zaventem, Belgium). Propiverine hydrochloride, M-5 (2,2-diphenyl-2-propoxy-acetic acid [1-methyl-piperid-4-yl]-ester-N-oxide-trans), M-6 (2,2-diphenyl-2-hydroxy-acetic acid [1-methyl-piperid-4-yl]-ester-N-oxide-trans), M-14 (2,2-diphenyl-2-propoxy-acetic acid [piperid-4-yl]-ester), and tamsulosin were synthesized at APOGEPHA Arzneimittel GmbH. Prazosin was from TOCRIS (Bristol, UK). Fluo-4 AM ester and pluronic acid were from Molecular Probes (via Invitrogen, Breda, The Netherlands). Phentolamine, phenylephrine, and all other chemicals were from SIGMA–ALDRICH (Taufkirchen, Germany).

Results

Effects on α_1-adrenoceptor-mediated prostate contraction

At the end of the equilibration period, human prostate strips exhibited a passive tension of 0.06 ± 0.01 mN/mg wet weight ($n = 16$ strips/five patients). Phenylephrine (10 μM) increased force of contraction to peak values of 0.16 ± 0.02 mN/mg ($n = 19/6$), which stabilized at steady-state values of 0.10 ± 0.02 mN/mg ($n = 16/5$) within 45 min (Fig. 1a). The maximum relaxation induced by 10 μM forskolin was 0.07 ± 0.01 mN/mg ($n = 16/5$). Propiverine, tamsulosin, and prazosin relaxed phenylephrine-induced contractions in a concentration-dependent manner (Fig. 1b).

Effects on α_1-adrenoceptor-mediated trigone contraction

Phenylephrine concentration-dependently contracted adult porcine trigone (but not bladder wall) with maximum contractions of 1.24 ± 0.23 mN/mg wet weight and a $-\log EC_{50}$ [M] of 5.74 ± 0.04 ($n = 6$). In juvenile tissues,

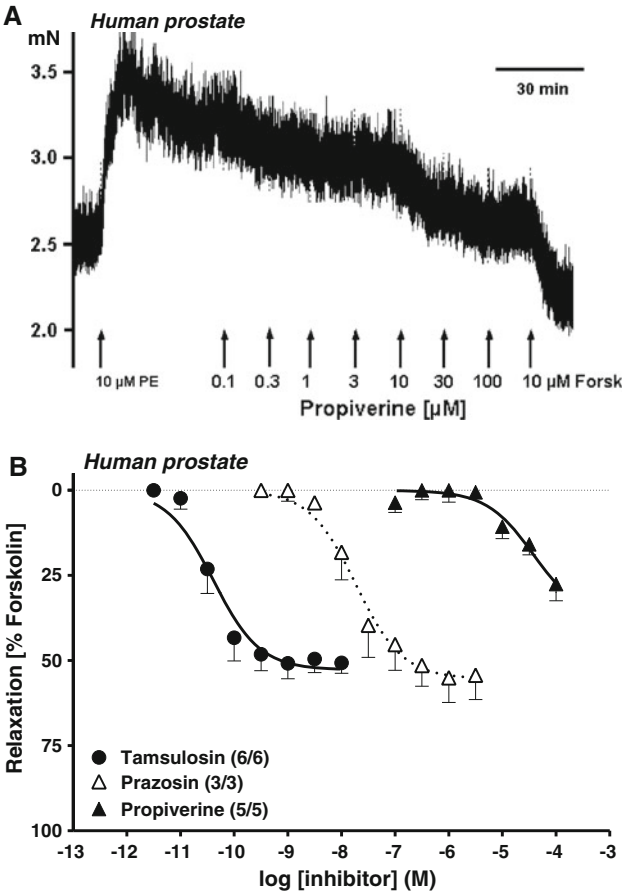

Fig. 1 **a** Original recordings of force of contraction in a human prostate tissue strip. The preparation was pre-contracted with phenylephrine (PE, 10 μM) (*left arrow*). After stabilization of force, propiverine or vehicle were added in increasing concentrations (0.1–100 μM). Finally, forskolin (10 μM) was added for complete relaxation. The difference between force prior to addition of the test compound and force in the presence of forskolin was taken as maximum relaxation (=100%). **b** Effects of tamsulosin, prazosin, and propiverine on α_1-adrenoceptor-mediated contractions in the human prostate (calculated –log IC_{50} values 10.39 ± 0.04, 7.73 ± 0.04, and 4.43 ± 0.08, respectively). Values were corrected for spontaneous relaxation during time-matched controls, normalized to percent relaxation by forskolin and expressed as means ± SEM

these contractions were concentration-dependently reversed by tamsulosin, prazosin, and propiverine (Fig. 2a). The overall relaxing effect of propiverine on contractions was similar in tissue from mature and juvenile animals, but the potency was lower in mature than in juvenile pigs ($-\log IC_{50}$ [M] 5.01 ± 0.05 vs. 6.21 ± 0.10, respectively; $n = 5$–7; $P < 0.05$; Fig. 2a,b). In trigone from mature pigs M-14 caused similar relaxation when compared to propiverine but with lower potency (4.84 ± 0.08; $n = 8$; Fig. 2b). M-5 and M-6 did not influence trigone contractions in concentrations up to 100 μM (data not shown).

Concentration-response curves for phenylephrine in porcine trigone strips in the presence of increasing

propiverine concentrations demonstrated that inhibition was largely insurmountable, i.e., mainly consisting of reduced maximum responses with only minor if any reductions in apparent potency (Fig. 3).

Binding to α_1-adrenoceptor subtypes

Direct α_1-adrenoceptor inhibition was evaluated in radioligand binding assays with CHO cells. Propiverine, M-14, and phentolamine competed for [^{3}H]-prazosin binding with −log K_i values of 4.72 ± 0.01, 4.72 ± 0.04, and 8.62 ± 0.19 at α_{1A}-, 4.94 ± 0.02, 5.02 ± 0.11 and 7.96 ± 0.22 at α_{1B}-, and 4.73 ± 0.02, 4.57 ± 0.06 and 7.87 ± 0.04 at α_{1D}-adrenoceptors, respectively (Fig. 4). In contrast, the metabolites M-5 and M-6 had little effect at up to 100 μM.

α_{1A}-Adrenoceptor-mediated intracellular Ca^{2+} elevation

The $[Ca^{2+}]_i$ increase with 10 μM phenylephrine was about 1,600 nM, and all subsequent data values are normalized to that response as measured within a given experiment (=100%). Propiverine and M-14 concentration-dependently inhibited the $[Ca^{2+}]_i$ elevations, but this inhibition largely consisted of reduced maximum responses with small if any effects on phenylephrine potency (Fig. 5a,b). M-5 or M-6 had little effect (Fig. 5c), whereas antagonism by phentolamine was surmountable (Fig. 5d).

Discussion

Propiverine differs from most other OAB drugs as it is not only a muscarinic receptor antagonist [5, 6] but also inhibits L-type Ca^{2+} channels [9–13]. Based upon the increasing interest in a combination treatment of male LUTS [14–16], we have explored possible α_1-adrenoceptor antagonism of propiverine and three of its main metabolites. The α_1-adrenoceptor antagonists phentolamine, prazosin, and tamsulosin were used as reference compounds and exhibited the expected potency for interaction with α_1-adrenoceptors, thereby validating our model systems and

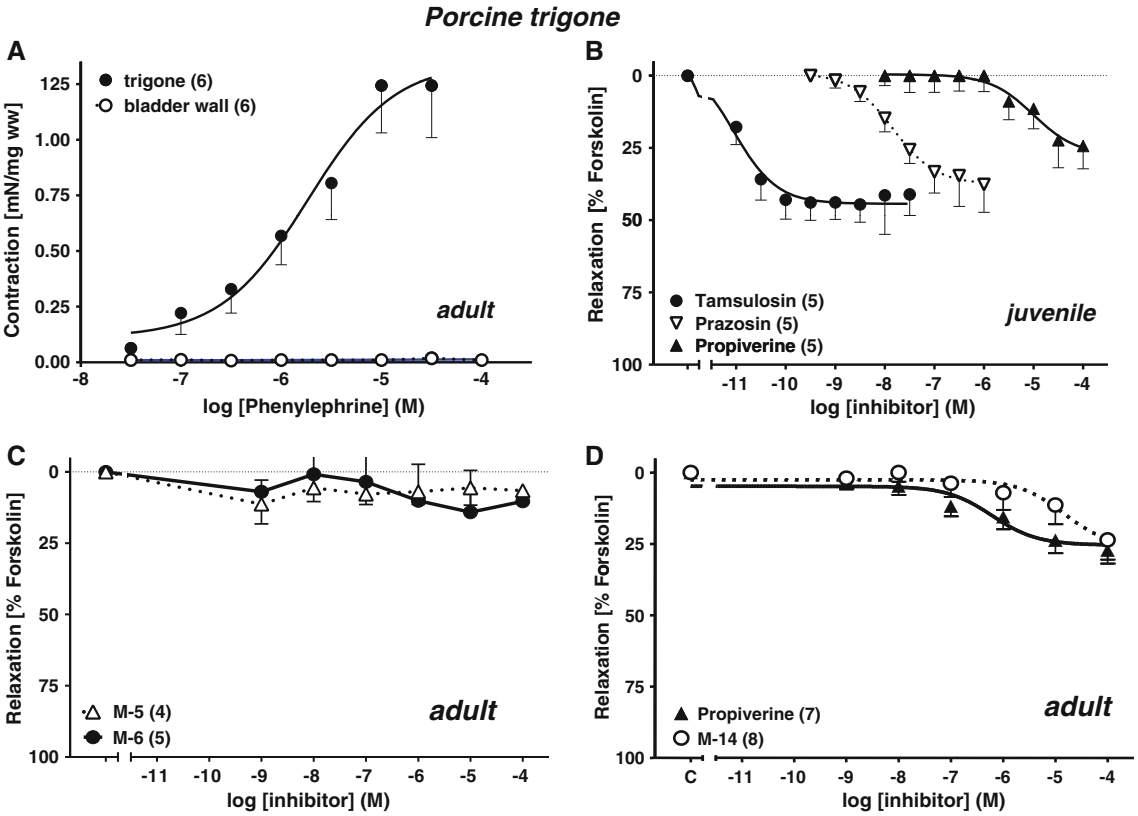

Fig. 2 **a** Effects of cumulatively added concentrations of phenylephrine on adult pig detrusor tissue from the trigone and the wall area of the urinary bladder. Data were normalized to mN/mg wet weight of the detrusor strip. **b** Effects of increasing concentrations of propiverine in comparison to those of the α_1-adrenoceptor antagonists tamsulosin and prazosin on juvenile porcine detrusor strips from the trigone area of the urinary bladder. Strips were pre-contracted with 10 μM of the α_1-adrenoceptor agonist phenylephrine. **c** Effects of increasing concentrations of propiverine and its metabolites M-5 and M-6 and **d** effects of propiverine and M-14 on trigone detrusor strips from mature pigs pre-contracted with phenylephrine (10 μM). Data in **b**–**d** were normalized to percent relaxation by forskolin (10 μM) and corrected for spontaneous relaxation during time-matched controls. Means ± SEM

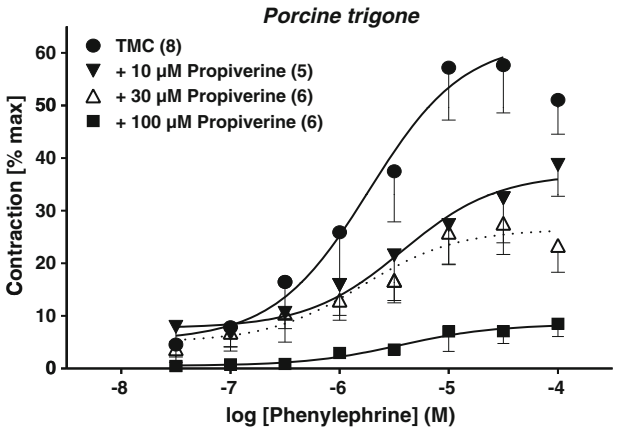

Fig. 3 Effect of increasing concentrations of propiverine on concentration-response curves for phenylephrine in adult porcine detrusor strips from the trigone area. Each strip underwent one phenylephrine curve in the absence of propiverine, and the result maximum response was defined as 100%. Then, phenylephrine response curves were repeated in the absence (time-matched control, TMC) and presence of 10, 30, and 100 μM propiverine yielding phenylephrine potencies ($-\log EC_{50}$) of 5.82 ± 0.19, 5.46 ± 0.38, 5.48 ± 0.28, and 5.90 ± 0.22, respectively. Corresponding Eff_{max} values were 58 ± 9, 39 ± 6, 28 ± 6*, and 9 ± 2** (expressed as % of values in corresponding first phenylephrine curve; * and **: $P < 0.05$ and < 0.01, respectively, vs. TMC). Means ± SEM from 5–8 experiments

techniques. Due to limited access to human prostate specimen for organ bath experiments as well as smaller signal/noise ratios, we have used porcine trigone as a well established animal model of α_1-adrenoceptor-mediated contraction of urological tissue for more detailed analysis.

Our data demonstrate concentration-dependent relaxation of phenylephrine-induced tone of human prostate and porcine trigone. At least in trigone, similar relaxation was observed with the propiverine metabolite M-14 but not with M-5 or M-6. As phenylephrine-induced contraction in these two preparations is α_1-adrenoceptor-mediated [17], this relaxation provided initial evidence for α_1-adrenoceptor antagonism by propiverine and M-14. However, L-type Ca^{2+} channels contribute to prostate and bladder contraction [9, 10]. The direct interaction of propiverine and its metabolites with α_1-adrenoceptors was demonstrated in competition binding studies with cloned human α_1-adrenoceptor subtypes, yielding affinities in line with their potency to relax human prostate and porcine trigone. To verify that the inhibition of radioligand binding was indeed associated with receptor antagonism, concentration-dependent inhibition of phenylephrine-induced $[Ca^{2+}]_i$ elevation was demonstrated. In contrast to tissue relaxation experiments, inhibition of $[Ca^{2+}]_i$ elevation in CHO cells cannot be explained by Ca^{2+} channel-blocking properties of propiverine, because CHO cells do not express such L-type Ca^{2+} channels. Together these experiments demonstrate that propiverine and its metabolite M-14 bind to

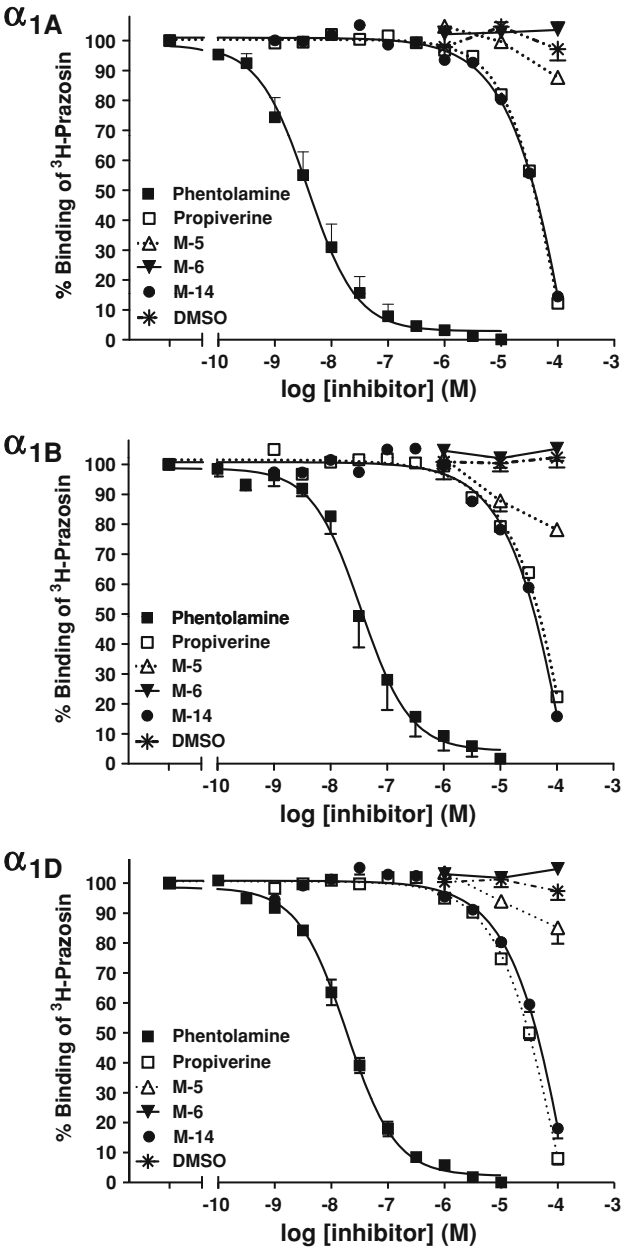

Fig. 4 Effects of propiverine and its metabolites M-5, M-6, and M-14 on [^{3}H]-prazosin binding in human α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptor-expressing CHO cells. The α_1-adrenoceptor antagonist phentolamine was used for comparison. Data in the presence of increasing concentrations of test compound are expressed in percent binding of [^{3}H]-prazosin. For control experiments only DMSO was added as a vehicle. Means ± SEM from 3 experiments

and inhibit human α_1-adrenoceptors and accordingly can relax α_1-adrenoceptor-mediated contraction of human prostate and porcine trigone.

Propiverine is a competitive antagonist with similar affinity for all muscarinic receptor subtypes [5, 6, 11]. However, the antagonism of $[Ca^{2+}]_i$ elevations in CHO cells and of contraction in porcine trigone was

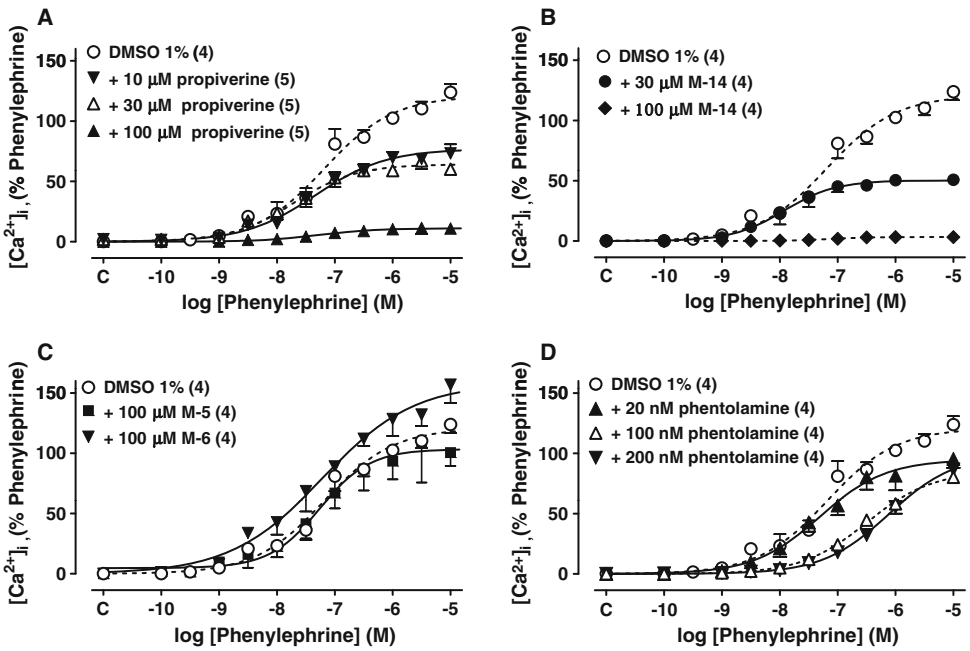

Fig. 5 Phenylephrine-stimulated elevation of $[Ca^{2+}]_i$ in α_{1A}-adrenoceptor-expressing CHO cells. **a** Control concentration-response curves in the presence of DMSO, and responses to increasing concentrations of propiverine. The vehicle DMSO did not significantly affect the efficacy or potency of phenylephrine to raise $[Ca^{2+}]_i$ ($n = 4$), data not shown. **b** Responses to M-14 and **c** responses to M-5 and M-6. **d** Responses to phentolamine. Data were normalized to $[Ca^{2+}]_i$ increase in the presence of 10 µM phenylephrine (=100%). Means ± SEM from 4–5 experiments

insurmountable. Thus, the molecular interaction of propiverine with α_1-adrenoceptors and muscarinic receptors occurs in a different way despite the drug being an antagonist for both receptor families. This differential interaction is also supported by the fact that the propiverine metabolites M-5 and M-6 lacked α_1-adrenoceptor effects but inhibit muscarinic receptor function [5, 10, 18, 19].

The affinity of propiverine for α_1-adrenoceptors in the present study differs from that for human M_3 receptors by 50- to 100-fold [5, 6, 12] but is very similar to its potency for inhibition of L-type Ca^{2+} channels [12]. As muscarinic antagonists typically are dosed to yield high receptor occupancy rates [20], it can be expected that therapeutic doses of propiverine exhibit some degree of α_1-adrenoceptor antagonism and L-type Ca^{2+} channel blockade. Based upon reported plasma concentrations of propiverine (parent compound alone) [21] and our affinity estimates from the radioligand binding studies, therapeutic propiverine concentrations could occupy up to 10% of α_1-adrenoceptors. While this may be less pronounced than the blockade of muscarinic receptors, it may nevertheless contribute to the clinical profile of propiverine and specifically may be beneficial for the treatment of male LUTS. Moreover, the insurmountable antagonism at α_1- (but not muscarinic) receptors raises the possibility that even limited α_1-adrenoceptor occupancy will yield considerable inhibition over time. Interestingly, a large recent observational study reported that the clinical effects of propiverine against OAB symptoms were quantitatively similar in men when administered alone or as add-on to an existing α-blocker treatment [22]. While these findings do not prove α_1-antagonism of propiverine in vivo, they are in line with this proposal. However, dedicated studies will be required to determine the clinical relevance of such effects. They should also take into account that propiverine and its various metabolites differ in their in vivo plasma concentrations [23]. For each of them, the relative contribution of the three molecular targets may differ in the generation of bladder selectivity and the overall relaxing effect in the lower urinary tract [24]. Even if propiverine itself turns out to have too little α_1-antagonism in vivo, it is an exciting starting point for the future synthesis of balanced α_1/muscarinic receptor antagonists.

Conclusions

In contrast to other drugs used for the treatment of OAB, propiverine is not only a muscarinic receptor antagonist but also has L-type Ca^{2+} channel blocking and α_1-adrenoceptor antagonist effects. While each of these effects may be beneficial in the treatment of voiding dysfunction, including male LUTS, the relative contribution of these mechanisms and of the propiverine metabolites to the overall therapeutic effects upon oral administration of propiverine remains to be determined.

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Conflict of interest The other authors do not report a conflict of interest other than those listed under Acknowledgments.

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