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Short communication

Juvenile pig detrusor: Effects of propiverine and three of its metabolites

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Abstract

In isolated detrusor strips, propiverine is known to be effective to decrease contractions elicited by electric field stimulation (EFS). Here we investigated whether the metabolites M-5, M-6 and M-14 of propiverine retain the pharmacological properties of the parent compound also in juvenile organisms. EFS-induced contractions of detrusor strips from juvenile pigs are more sensitive to atropine than strips from mature pigs. The atropine-resistant component of contraction is also significantly larger in juvenile pigs. Propiverine, its metabolites M-5, M-14 and also tolterodine completely reduced detrusor contraction in juvenile pigs. M-6 almost did not affect atropine-resistant contractions. We conclude that juvenile pig detrusors possess a higher atropine-resistant component of EFS-elicited contraction. Nevertheless order of potency and efficacy of propiverine and its metabolites M-5 and M-14 are similar in juvenile and mature pigs, while M-6 only reduces atropine-sensitive contractions in the juvenile organism.

Keywords: Juvenile pig; Detrusor contraction; Propiverine and metabolites

1. Introduction

Antimuscarinic drugs are commonly prescribed for treatment of the overactive bladder syndrome (Sellers et al., 2001; Andersson et al., 2002; Andersson and Yoshida, 2003; Ouslander, 2004). Among these, propiverine appears to have other spasmolytic effects in addition to its antimuscarinic actions (Andersson et al., 1999; Wuest et al., 2002). During clinical use, monotherapy with propiverine was shown to be effective in children with urge syndrome and urge incontinence (Marschall-Kehrel et al., 2004).

In adult human detrusor, propiverine (i) completely blocks contractile responses elicited by electric field stimulation (EFS) including the atropine-resistant component; (ii) impairs contractions induced by KCl or $CaCl_2$ and (iii) suppresses the maximum responses to acetylcholine in addition to shifting the concentration–response curve to the right (Wada et al., 1995). In guinea-pig bladder strips, propiverine also reduces KCl-induced contractions (Haruno, 1992; Tokuno et al., 1993) and in rabbit detrusor, the drug impairs intracellular Ca^{2+}-homeostasis in addition to its antimuscarinic effects (Madersbacher and Mürtz, 2001).

Propiverine is rapidly absorbed after oral administration and is subject to extensive first-pass metabolism giving rise to several active metabolites such as 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) and M-14 (2,2-diphenyl-2-propoxy-acetic acid [piperid-4-yl]-ester). Twenty-four hours after ingestion, the parent and its metabolites can be recovered from the human urine in the following amount (percent of original dose): propiverine, 2–3%; M-5, 20%; M-6, 5%; and M-14, 1% (Haustein and Hüller, 1988).

Recently we reported that propiverine and the three metabolites reduce EFS-evoked detrusor contractions, albeit to different extents. For the spasmolytic effects the order of potency was: M-6 > propiverine ≈ M-14 > M-5 (Wuest et al., 2002, 2005). Like propiverine, M-5 and M-14 suppress the atropine-resistant component of contraction and reduce the maximum effects of the carbachol–concentration response curve, but this response pattern is not shared by M-6 (Wuest et al., 2002, 2005).

Presently, little is known about antimuscarinic drug sensitivity and efficacy in young organisms. Longhurst (2004)

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reported that juvenile pig detrusor was more sensitive to atropine than in tissue from adult animals. However, the pharmacodynamic effects of propiverine and its metabolites have not been investigated before in tissue from juvenile animals. Therefore we have studied the potency and efficacy of propiverine and three of its metabolites in detrusors from young pigs, which are generally considered to be similar to human tissue. The standard antimuscarinic agent atropine and the spasmolytic drug tolterodine were included for comparative purposes.

2. Methods and materials

2.1. Juvenile pigs

Urinary bladders of juvenile pigs (8–12 weeks) were obtained from a local abattoir and transported within 30 min to the laboratory. All experiments were in accordance with the European Community Guidelines for the use of experimental animals. Muscle preparations from 9 male and 3 female pigs of 12 to 35 kg body weight were used. Suitable muscle strips of urothelium denuded detrusor (4–8 strips 7–10 mm long, 1–3 mm wide) were dissected as previously reported (Wuest et al., 2002). The mean weight of the muscle strips was 73±5 mg (n=51).

2.2. Detrusor muscle contractions

Each detrusor strip was mounted in a 25 ml organ baths with oxygenated Tyrode's solution (Wuest et al., 2002). Contractions were measured with an isometric force transducer (GM 2, Föhr Medical Instruments, Seeheim/Ober Beerbach, Germany). Resting load was set to 10 mN and readjusted after 30 min. During the equilibration period of 60 min the bath solution was changed once. Detrusor strips were incubated twice with 1 μM carbachol for 10 min each with a 15 min wash-out period between exposures. After an additional 20 min of stabilization muscle strips were subjected to EFS with the following parameters: pulse duration 1 ms, amplitude 90 mA, frequency 30 Hz, trains of stimuli for 5 s and 2 min between the trains of stimuli. The compounds under investigation were added in cumulatively increasing concentrations with 30 min between increments. To verify changes of the muscle contractions during the entire experimental period control experiments were performed in the absence of any drug added (time-matched controls). At the end of each experiment the neurotoxin tetrodotoxin (1 μM) was added to quantify the non-neuronally mediated amount of muscle contraction.

2.3. Statistics

Average values for the EFS-induced muscle contraction amplitudes were obtained from the 5 contractions preceding the next concentration increment. The magnitude of drug effect is given in percent inhibition of the EFS-evoked contraction amplitude before any drug addition (=100%). All data are expressed as means±S.E.M. Individual IC_{50} (molar drug concentration producing 50% inhibition of maximum contractile response to EFS) were determined by nonlinear regression analysis for each individual experiment using GraphPad Prism® 3.02 (GraphPad Software Inc. San Diego, USA). Mean IC_{50} values are expressed as $-\log IC_{50}$ [mol/l] ±S.E.M. from n experiments. To evaluate differences in the effects of all compounds between juvenile and mature pig Student's t-tests were performed and differences were considered significant for a $P<0.05$.

2.4. Drugs and solutions

Carbachol, atropine sulfate and tetrodotoxin acetate were purchased from SIGMA-ALDRICH; propiverine hydrochloride, M-5, M-6, M-14 and (*R*)-tolterodine-L (+)-tartrate were obtained from APOGEPHA Arzneimittel GmbH, Dresden, Germany. Carbachol and atropine were dissolved in water. For all other compounds dimethyl sulfoxide (DMSO) was used as solvent for the 0.1 mol/l stock solution. All dilutions were made with Milli-Q-water. The maximum DMSO concentration in the aqueous solutions of the organ bath was 1%. This DMSO concentration did not cause any significant effects on the muscle contraction under our experimental conditions. The Tyrode's solution (gassed with carbogen; pH=7.4) contained (mmol/l): 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.

3. Results

All detrusor strips were exposed twice with 1 μM carbachol before starting electric field stimulation. Fig. 1A depicts the effects on electrically evoked contractions of atropine, propiverine and M-14. Fig. 1B summarizes the concentration–response curves for M-6, M-14 and atropine, while Fig. 1C displays the analogous curves for propiverine, its *N*-oxide M-5 and tolterodine (time-matched controls are shown in both figures). The calculated concentrations for the half-maximum inhibition ($-\log IC_{50}$) and the maximum inhibition ($Inhib_{max}$) are summarized in Table 1. When the $-\log IC_{50}$ values were compared, the following order of potency was found: atropine>M-6 ≈ tolterodine>propiverine ≈ M-5 ≈ M-14.

EFS-evoked contractions remained constant over the time course of the experiment. After 4 h tension was still 95±11% of control (n=11). In juvenile pig detrusor, atropine (10 μM) reduced electrically evoked contractions to 44±9% of control (n=7/7) whereas the same concentration depressed EFS-contractions to 20±2% of control (n=8) in mature pigs (Wuest et al., 2002). In juvenile pigs M-6 was about two orders of magnitude less potent than atropine but it depressed detrusor contractions to the same extent. M-5 and M-14 reduced detrusor contractions with similar potency and efficacy as the parent compound propiverine (see Table 1). Tolterodine and M-6 displayed comparable potency like M-6, but tolterodine reduced electrically induced contractions to a greater extent. At

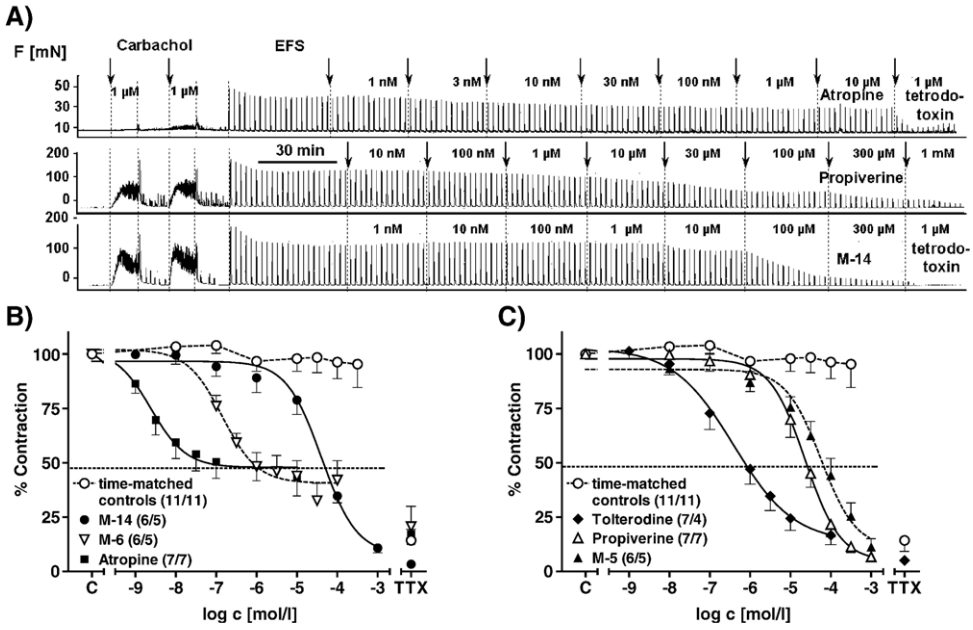

Fig. 1. A) Force of contraction elicited by electric field stimulation in 3 juvenile pig detrusor strips after strips were exposed twice to 1 µM carbachol. 1st to 3rd strip, responses to increasing concentrations of atropine, propiverine and M-14. In the absence of antagonists electrically induced forces decreased slightly. Contractile responses usually stabilized within 45 min. The weights of the individual strips were (from top to bottom): 113, 68 and 89 mg w.w., respectively. For clarity calculated concentration–response curves are presented in two different diagrams: B) Concentration-dependent effects of M-6, M-14 and atropine on electrically stimulated force development in juvenile pig detrusor muscle strips in comparison to time-matched control recordings. C) Effects of propiverine, M-5 and tolterodine on electrically stimulated force development also in comparison to time-matched control recordings. Contractions in the presence of a given drug concentration are expressed in percent of the pre-drug control value (=100%). Data are presented as means±S.E.M. of *n* investigated strips from × different animals.

100 µM the maximum inhibition effect reached 83±4% of control ($n=7$).

Adding 1 µM of the neurotoxin tetrodotoxin at the end of the time-matched control experiments resulted in reduction of contraction amplitudes to 14±5% of control ($n=11$). This residual component contained the non-neuronal mediated detrusor contractions, which are of myogenic nature. Administration of tetrodotoxin in conjunction with the maximum atropine concentration further reduced EFS-induced contractions to 17±5% of control indicating an atropine-resistant component of contraction of about 27% under our experimental conditions.

Table 1
Comparison of half maximum inhibition (−log IC_{50}; mol/l) and the maximum inhibition ($Inhib_{max}$; %) of each investigated compound in *juvenile* and *mature* pigs

Compound	*n*	Juvenile pigs		*n*	Mature pigs [d]	
		−log IC_{50}	$Inhib_{max}$		−log IC_{50}	$Inhib_{max}$
		[mol/l]	[%]		[mol/l]	[%]
Atropine	7	8.49±0.14 [a]	56±9 [b]	18	7.82±0.08 [a]	80±2 [b]
Propiverine	7	4.76±0.24	93±1	8	4.73±0.12	90±1
M-5	6	4.14±0.21	89±3	6	3.90±0.15	95±1
M-6	6	6.85±0.13	58±7 [c]	14	6.26±0.03	81±3 [c]
M-14	6	4.23±0.08	89±2	11	4.24±0.23	71±5
Tolterodine	7	6.35±0.21	83±4	7	5.95±0.64 [e]	88±2 [e]

Data as means±S.E.M. of *n* investigated muscle strips.
[a] significant difference $P<0.01$.
[b] $P<0.001$.
[c] significant difference $P<0.01$.
[d] data from Wuest et al., 2005.
[e] data from Wuest et al., 2002.

4. Discussion

Our results indicate that the size of the atropine-resistant component of contraction is larger in juvenile than in mature pigs. We confirm that atropine is significantly more potent in strips from juvenile (−log $IC_{50}=8.49\pm0.14$, $n=7$) than from mature pigs (7.82±0.08, $n=18$, $p<0.001$; Wuest et al., 2002). The signal transduction via muscarinic receptors is fully developed at birth and does not change during maturation. On the other hand ATP and noradrenaline mediated contractile responses are thought to be developmentally regulated (Longhurst, 2004). The observed atropine-resistant part of contraction was about 4 times larger in juvenile detrusor compared to values obtained in adult animals (Wuest et al., 2002). Our data with atropine and tetrodotoxin suggest that the non-cholinergic neurotransmitter mediated contribution to contractile responses after EFS is larger in juvenile pigs and probably decreases during functional maturation. In adult animals the EFS-evoked contractions are mainly mediated by neuronal release of acetylcholine (Sibley, 1984). Thus detrusor responses in juvenile pig bladder resemble those of the adult mouse bladder, atropine-resistant contractions amounted to 70% of control (Wuest et al., 2002). Potency and efficacy of propiverine, M-5, M-14 and also tolterodine are similar in detrusor strips from juvenile and mature pigs (see Table 1).

Effects of M-6 in juvenile pigs are similar to the findings in mouse detrusor in which M-6 reduced contractions to 50% of control and had no influence on atropine-resistant contractions (Wuest et al., 2005).

We conclude that the juvenile pig detrusor possesses a higher atropine-resistance and therefore a higher non-cholinergic mediated component of EFS-elicited contraction. Nevertheless the effects of propiverine, its metabolites M-5, M-6 and M-14 and also tolterodine (order of potency and maximum inhibition of contractions) are similar in juvenile and mature pigs.

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