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GASTROINTESTINAL FUNCTION

Muscarinic receptor expression and receptor-mediated detrusor contraction: comparison of juvenile and adult porcine tissue

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Abstract Urinary bladder function is known to mature during fetal and postnatal development, including changes in neurotransmitter regulation of detrusor contraction. However, only few experimental data are available about muscarinic receptor antagonist function in the urinary bladder from young animals. In the present study, we compare the muscarinic receptor-mediated contractions in juvenile and adult porcine detrusor and the effects of antimuscarinic compounds. Urinary bladders from young (8–12 weeks; 12- to 35-kg body weight) and mature pigs (>40 weeks; >100 kg) were compared. Muscarinic receptor expression was assessed by real time polymerase chain reaction and radioligand binding. Muscle contraction was measured with a force transducer; L-type Ca^{2+} currents ($I_{Ca,L}$) of isolated detrusor myocytes were recorded with standard voltage clamp technique. Juvenile and adult detrusor expressed similar quantities of the messenger RNA of M_2 and M_3 receptors. The number of [^{3}H]QNB-binding sites and their affinity for the radioligand were also similar between juvenile and adult detrusor. In contrast, maximum contractile responses to the muscarinic receptor agonist carbachol were slightly larger in juvenile than adult bladders. On the other hand, carbachol was slightly less potent in juvenile than in adult tissue. The M_3 antagonist DAU 5884 and the spasmolytic drug propiverine inhibited contractile responses with comparable efficacies and potencies in juvenile and adult tissue. $I_{Ca,L}$ was somewhat smaller in juvenile than in adult cells. Taken together, these data suggest that expression and function of M_2 and M_3 receptors are similar in the detrusor of juvenile and mature pigs. Therefore, similar responses to antimuscarinic compounds could be expected in young and adult patients.

Keywords Urinary bladder · Muscle contraction · Muscarinic receptor · Ca^{2+} influx · Ca^{2+} current · Propiverine

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Introduction

Smooth-muscle contraction in the urinary bladder is mainly controlled by muscarinic acetylcholine [M] receptors [2, 14]. Although M_2 receptors are the predominant receptor subtype found in detrusor, M_3 receptors were shown to play a key role in mediating contractile function [1, 6, 9, 14, 28, 40]. After activation of M_3 receptors, the intracellular Ca^{2+} level increases by both intracellular Ca^{2+} release and Ca^{2+} influx via L-type Ca^{2+} channels. The latter process is thought to be essential for detrusor contraction because force development is diminished in Ca^{2+} channel ($Ca_v1.2$) knockout mice [35] or in the presence of the L-type Ca^{2+} channel blocker nifedipine [22, 28, 38]. Thus, muscarinic receptor-induced detrusor contractions involve Ca^{2+} entry via L-type Ca^{2+} channels followed by uptake of Ca^{2+} into intracellular stores

and a subsequent release either via IP_3-dependent or Ca^{2+}-induced processes via ryanodine receptors [7, 10, 26].

Muscarinic receptor-mediated contractions and their underlying cellular signal transduction pathways during early development have been studied in different animal models (for review see [19]). Fetal detrusor contracts in response to muscarinic receptor agonist stimulation, indicating the presence of functionally active muscarinic receptors. In a rat study, neonatal bladder tissue was significantly more sensitive to agonistic stimulation of either muscarinic or purinergic receptors than tissue from 1- or 4-month-old rats, indicating dramatic changes during prenatal but only minor alterations in postnatal development; relaxation responses to isoprenaline did not differ substantially between the three groups [32]. Studies comparing fetal calf bladders at different developmental stages suggest that all components required for muscarinic receptor stimulation are mainly developed at birth and hardly change during maturation [16]. On the other hand, adenosine triphosphate (ATP)- and noradrenaline-mediated contractile responses appear to be regulated during postnatal development [24, 41] and are related to maturation of Ca^{2+} influx and cellular storage of Ca^{2+} [42]. Recently, we found that atropine-resistant contractions are larger in juvenile than adult porcine detrusor, suggesting a strong component of noncholinergic contraction in juvenile tissue [36]. This supports earlier findings of decreasing contribution of nonadrenergic, noncholinergic contractions during maturation [19].

Antimuscarinic compounds are the most widely used drugs for treatment of overactive bladder [3, 25]. Clinically employed agents include nonselective and M_3-selective receptor antagonists, including drugs with additional spasmolytic effects [1, 14]. Propiverine possesses both antimuscarinic and direct spasmolytic effects [37]. The basis for these spasmolytic effects appears to be inhibition of L-type Ca^{2+} currents ($I_{Ca,L}$) in detrusor smooth-muscle cells from different species [33, 37] and its ability to displace selective L-type Ca^{2+} channels blockers from their binding sites within the channels [34]. Accordingly, propiverine inhibits not only contractions elicited by electric-field stimulation or M receptor agonists but also those induced by KCl or $CaCl_2$ ([36]; for review, see [20]).

Worldwide, only two drugs, oxybutynin and propiverine, are approved for the treatment of overactive bladder (OAB) or neurogenic detrusor overactivity in children. While several clinical trials in children with antimuscarinic compounds were published, the response to placebo was rather high. Recently, a well-designed randomized and placebo-controlled study was presented, showing efficacy and safety of propiverine in the treatment of OAB in children aged 5 to 10 years [21]. Further research of the physiological function of the lower urinary tract during postnatal development and more clinical studies [15, 23, 31] are necessary to consolidate the available treatment options for children suffering from these conditions.

In the present study, we have explored differences between juvenile and adult detrusor function. For this purpose, we compared isolated detrusor tissues from juvenile and adult pigs with respect to M receptor subtype expression and functional responses to the M_3-selective receptor antagonist DAU 5884 and the spasmolytic drug propiverine. In particular, we have studied the drug effects on contractile function and on L-type Ca^{2+} currents.

Materials and methods

Quantitative messenger RNA expression

Urinary bladders from juvenile (8–12 weeks; 12- to 35-kg body weight) and adult (>40 weeks; >100 kg) pigs were obtained from a local abattoir and transported to the laboratory within 1 h. Fresh detrusor smooth-muscle tissue was dissected free from the mucosa and serosa layers and then frozen immediately. Total RNA was extracted from frozen detrusor samples using the EZNA total RNA kit (Peqlab, Erlangen, Germany). Samples from 10–12 juvenile and 8–10 adult animals were analyzed. All samples were used free of protein and DNA contaminations. Real-time polymerase chain reaction (RT-PCR) was performed using the QuantiTect SYBR® Green RT-PCR kit (Qiagen, Hilden, Germany) and a Rotor Gene thermal cycler (Corbett Research, Mortlake, Australia). The following subtype gene-specific primers were used (5′→3′): muscarinic receptor subtype 2 (M_2) sense AAG AAG GAC AAG AAG GAG CC, antisense CTT TGG AAT GGC CCA GG; muscarinic receptor subtype 3 (M_3) sense AAC AAT GAT GCT GCT GCC, antisense GTG ATC TGA CTT CTG GTC TTC. RT-PCR, and RT-PCR conditions were 94°C for 5 min, 30 s at 94°C, followed by 30 s at 60°C and 40 s at 72°C, 72°C for another 7 min for M_2 receptors lasting 30 cycles, and 94°C for 5 min; and 30 s at 94°C, followed by 30 s at 54°C and 30 s at 72°C and 72°C for another 7 min for M_3 receptors lasting 39 cycles. Competitor RNA (internally deleted standard RNA) was produced by T7 in vitro transcription using the message machine kit (Ambion, Austin, TX, USA) from RT-PCR products produced as described above but with a modified sense primer containing an additional T7 promoter sequence (GGC CGC GG). Standards of known concentration of RNA included in each dilution series (10^5–10^{12} molecules/μl) allowed calculation of competitor RNAs. PCR efficiency was 85 to 92%. Results were analyzed by the Rotorgene software version 4.6 (Corbett Research). The amount of muscarinic messenger RNA (mRNA) was normalized to total mRNA. The identity of the PCR product was confirmed by sequence analysis as described previously [12].

Radioligand-binding studies

Muscarinic receptors were identified at the protein level by radioligand binding studies as previously described [29]. Briefly, muscarinic receptors were identified in saturation-binding studies using [^{3}H]-*l*-quinuclidinylbenzylate ([^{3}H]QNB) as the radioligand. Tissues were homogenized in ice-cold preparation buffer (50 mM Tris, 10 mM $MgCl_2$, 0.5 mM ethylenediaminetetraacetic acid, pH 7.5) with an Ultra-Turrax (Janke & Kunkel, Staufen, Germany). The homogenates were centrifuged for 20 min at 50,000×*g* at 4°C. The pellets were resuspended in buffer, rehomogenized shortly (10 s at full speed) and washed by an additional centrifugation step. The final pellets were resuspended and rehomogenized in binding buffer (10 mM Na_2HPO_4, 10 mM NaH_2PO_4 at pH 7.4). Binding experiments were performed in a total volume of 1,000 μl buffer containing 50–100 μg protein/assay. Incubations were performed for 60 min at 37°C and terminated by rapid vacuum filtration over Whatman GF/C filters. Each filter was washed four times with 5 ml each of ice-cold binding buffer. Nonspecific binding was defined by 3 μM atropine.

Detrusor contractions

Serosa- and mucosa-free porcine detrusor smooth-muscle tissue was mounted in 5-ml organ baths containing Tyrode's solution, which was maintained at 37°C and continuously gassed with 95% O_2 and 5% CO_2. Isometric tension was measured using force transducers from GM 2, Föhr Medical Instruments (Seeheim/Ober Beerbach, Germany), and recorded using ADI PowerLab Chart software. The tissues were subjected to a resting tension of 1 g and equilibrated for 60 min. Thereafter, cumulative concentration–response curves for carbachol were generated with 5 min of exposure to each concentration. Tissues were then washed for 60 min and incubated for another 60 min with a suitable concentration of the M_3 receptor-selective antagonist DAU 5884 (1–10 nM; [11]), propiverine (0.1–100 μM), or vehicle (time-matched controls), followed by generation of a second concentration–response curve for carbachol. The relaxing effects of nifedipine (1 nM–100 μM) were tested in detrusor strips precontracted by carbachol (1 μM) or KCl (40 mM). Tonic contraction after 45 min incubation with carbachol or KCl were considered as minimum relaxation (= 0%). Data were normalized to maximum relaxation (=100%) by 10 μM forskolin added at the end of each experiment.

Measurement of L-type Ca^{2+} currents ($I_{Ca,L}$)

Porcine detrusor smooth muscle cells were isolated according to our standard protocol [37] and immediately used for experiments. $I_{Ca,L}$ was measured at 21–23°C with standard voltage clamp technique (Axopatch 200, Axon Instruments, Foster City, CA, USA); ISO 2 software (MFK, Niedernhausen, Germany) was used for data acquisition and analysis. Tip resistances of heat-polished pipettes were 2–6 MΩ; seal resistances were about 1 GΩ. Cell capacitance (Cm) was calculated from steady-state current during depolarizing ramp pulses (1 Vs^{-1}) from −40 to −35 mV. $I_{Ca,L}$ was measured from a holding potential of −60 mV at a test potential of +10 mV. For further isolation of $I_{Ca,L}$ from contaminating currents, Na^+ was replaced with tetraethylammonium ions, and K^+ was replaced with Cs^+ to block K^+ currents. As charge carrier, 3.8 mM Ba^{2+} was used. Cell superfusion buffer contained (in millimolar) tetraethylammonium chloride, 120; CsCl, 10; 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES), 10; $BaCl_2$, 3.8; $MgCl_2$, 1; and glucose, 20; pH 7.4 (adjusted with CsOH). The pipette solution consisted of (in millimolar) cesium methanesulphonate, 90; CsCl, 20; HEPES, 10; Mg-ATP, 4; Tris–GTP, 0.4; ethylene glycol *bis*(2-aminoethyl ether)-*N*,*N*,*N*′*N*′-tetraacetic acid, 10; and $CaCl_2$, 3 (pH 7.2). Current amplitudes were determined as the difference between the peak inward current and current at the end of the depolarizing clamp step. A system for rapid solution changes (Cell Micro Controls, Virginia Beach, USA; ALA Scientific Instruments, Long Island, NY, USA) allowed application of substances in the close vicinity of the cells.

Statistical analysis

All data are expressed as means ± SEM. Cumulative concentration–response curves were analyzed by nonlinear regression of each individual experiment using GraphPad Prism® 3.02 (GraphPad Software, San Diego, CA, USA); mean values for molar concentrations producing half-maximum responses to carbachol pEC_{50} [M] were calculated from concentration–response curves before and after test-drug addition. Maximum contraction during the second concentration–response curve for carbachol (Eff_{max}) is expressed in percent of the maximum effects during the first concentration–response curve (=100%).

Statistical differences were tested by Student's *t* test and were considered significant for $p<0.05$. Dissociation constants (pK_B) for DAU 5884 were determined by Schild plot analysis [4].

Drugs

Carbachol (carbamoylcholine chloride) and nifedipine were obtained from Sigma-Aldrich (Taufkirchen, Germany). DAU 5884 (8-methyl-8-azabicyclo-3-endo[3.2.1]oct-3-yl-1,4-dihydro-2-oxo-3(2H)-quinazolinecarboxylic acid ester hydrochloride) was purchased from Tocris (Bristol, UK).

Propiverine hydrochloride was provided by APOGEPHA Arzneimittel GmbH (Dresden, Germany). [^{3}H]QNB (specific activity 48 Ci/mmol) was from Amersham (Braunschweig, Germany). All other chemicals were purchased from Sigma-Aldrich. Stock solutions were prepared in Milli-Q water and further diluted in the appropriate buffers.

Results

Quantitative mRNA expression

Quantitative expression of mRNA of M_2 and M_3 receptor subtypes was examined in detrusor samples from juvenile and adult pigs with RT-PCR. In adult porcine detrusor tissue, M_3 receptors were expressed at slightly higher levels than M_2 receptors: 32±12 (n=10) vs 18±5 fg/ng mRNA (n=8); however, this difference did not reach the level of statistical significance. Expression of M_2 and M_3 receptors was similar in juvenile and adult tissue (Fig. 1).

Radioligand binding

Muscarinic receptor protein levels were estimated from [^{3}H]QNB saturation binding experiments (Fig. 2). In juvenile and adult pigs, maximum binding of [^{3}H]QNB (B_{max}) was 36±5 fmol/mg protein (n=10) and 48±6 fmol/mg protein (n=10; n.s.), respectively. The dissociation constants K_D for radioligand binding were 7.3±2.3 pmol/l in juvenile and 8.0±1.9 pmol/l in adult tissue (n=10 each; n.s.).

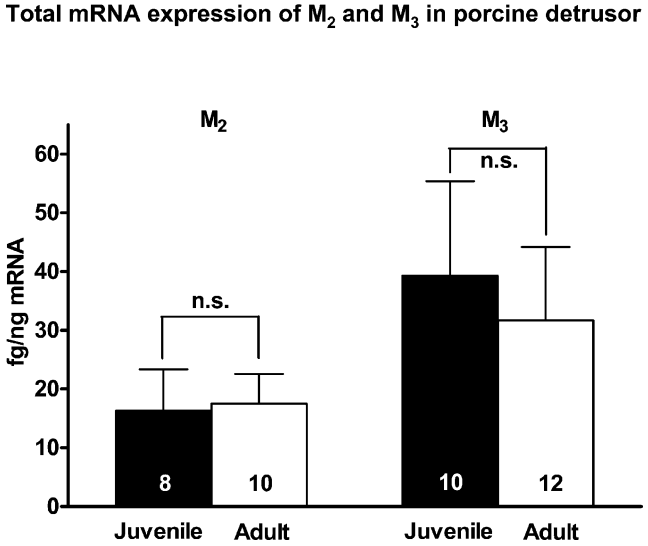

Fig. 1 Quantitative mRNA expression of M_2 and M_3 receptor subtypes in juvenile and adult porcine detrusor tissue. Data as mean + SEM from n different animal preparations. *n.s.* Not significant

Detrusor contraction

Force of contraction in response to increasing concentration of carbachol was significantly different in juvenile and adult tissues. When pooling data from the first concentration–response curve of all individual muscle strips from the investigated bladders (n number of strips/x animals), the mean maximum increases (Eff_{max}) in force of contraction were 2.03±0.23 mN/mg w.w. (juvenile, n=54/14) and 1.40±0.20 mN/mg w.w. (adult, n=52/14), respectively (p<0.05). The corresponding pEC_{50} [M] values were 5.52±0.06 (juvenile, n=54/14) and 5.80±0.06 (adult, n=52/14; p<0.01).

In time-matched controls (TMC), the second concentration–response curves for carbachol were shifted to higher concentrations, yielding pEC_{50} [M] values of 5.12±0.11 (n=16/14) and 5.48±0.13 (n=16/14) for juvenile and adult tissue, respectively. This indicated a rightward shift (ΔpEC_{50}) of 0.40±0.09 and 0.41±0.06 in the absence of any drug added. The pEC_{50} in TMC were used for the comparison of drug effects.

The contribution of M_3 receptors to carbachol-induced detrusor contractions was tested with the subtype-selective receptor antagonist DAU 5884 in a concentration range in which the compound is selective for M_3 receptor subtypes [11]. DAU 5884 (1–10 nM) shifted the concentration–response curve for carbachol to higher agonist concentrations in juvenile and adult detrusor strips (Fig. 3a,b). Although pEC_{50} values for carbachol in juvenile detrusor were slightly lower than in adult tissue, the actual shifts of the concentration–response curve by each individual concentration of DAU 5884 were similar. The pK_B values corrected for the shift in TMC experiments and estimated from Schild plot analysis (Fig. 3c) were also slightly but not significantly different, indicating that potency of DAU 5884 for muscarinic receptors mediating detrusor contraction from juvenile animals is similar to adult pigs.

The effects of propiverine on the concentration–response curves for carbachol are depicted in Fig. 4a,b. Increasing concentrations of propiverine not only shifted the concentration–response curves to the right but also reduced maximum carbachol responses (Eff_{max}). In juvenile and adult tissue, propiverine shifted the pEC_{50} values for carbachol to a similar extent, although the variability of individual results was rather large (Table 1). The propiverine concentrations for half maximum suppression of force of contraction were calculated from the impaired maximum responses to carbachol and were expressed as pIC_{50} [M] values. They were 4.97±0.15 (n=4–11) in juvenile and 5.19±0.35 (n=3–9; p>0.05) in adult tissue, suggesting similar relaxing potency of propiverine in juvenile and adult detrusor.

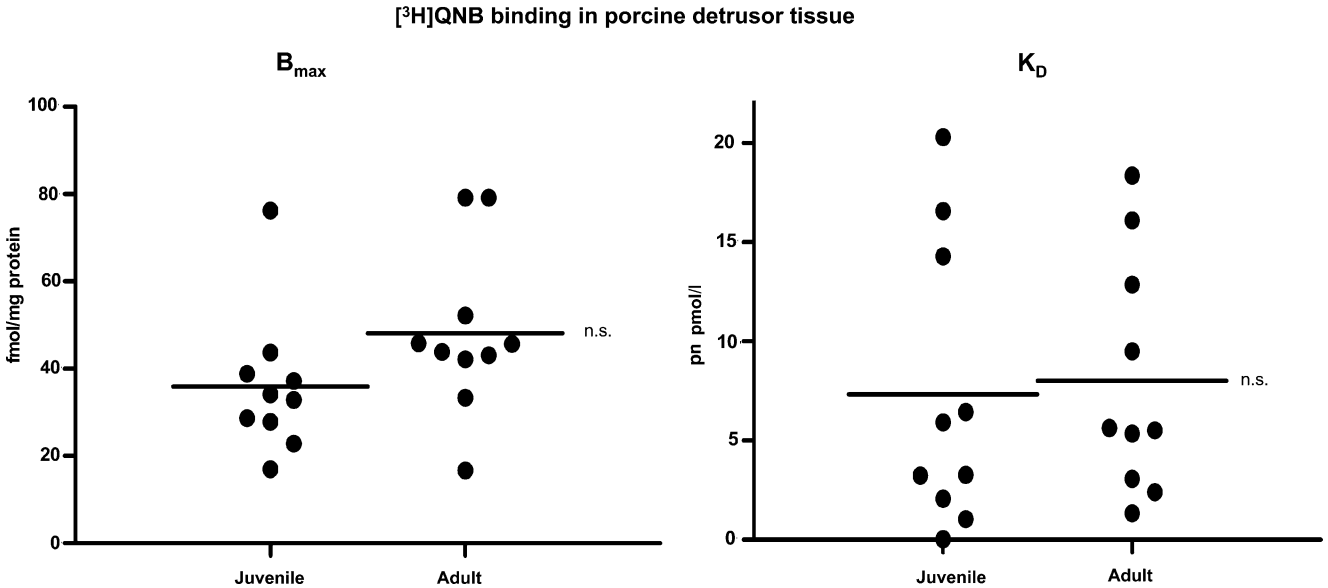

Fig. 2 Binding of [^{3}H]QNB in detrusor tissue from juvenile and adult pigs. Maximum binding of [^{3}H]QNB (B_{max}) and affinity dissociation constants K_D are presented. Data are shown as single points from individual determinations. The mean value from all single points is indicated as a *line*

L-type Ca^{2+} currents

Potential contribution of Ca^{2+} influx to porcine detrusor contraction was investigated in precontracted muscle strips exposed to increasing concentrations of nifedipine. (Fig. 5a,b). Increasing the extracellular K^+ concentration will depolarize the smooth muscle and neuronal cells within the muscle strips. Therefore, atropine (1 μM) was added to suppress contribution of any pre- or postsynaptic M receptors. Under such conditions, addition of 40 mM KCl produced a sustained increase in force of contraction that could be almost completely abolished by increasing concentrations of nifedipine. Stimulation of adenylyl cyclase with forskolin (10 μM) completely reversed KCl-induced contractions in both age groups. The potency of nifedipine (pIC_{50} [*M*]) was 7.82±0.21 (n=6/4) in juvenile versus 7.56±0.33 (n=8/6) in adult detrusor strips (n.s.). Contractile force in muscle strips precontracted with 3 μM carbachol also stabilized after a transient increase. In this system, nifedipine was one order of magnitude less potent as a relaxant than in KCl-induced contractions. The pIC_{50} [*M*] values for nifedipine were 6.55±0.28 (n=5/5) in juvenile and 6.37±0.21 (n=5/5) in adult tissue (n.s.). Maximum responses to nifedipine were similar in juvenile and adult tissue.

Finally, L-type Ca^{2+} current ($I_{Ca,L}$) was studied directly in freshly isolated detrusor smooth-muscle cells. Membrane capacitances of juvenile and adult detrusor cells were similar, i.e., 44±4 pF and 50±4 pF (n=14/6 each, n.s.), suggesting similar cell sizes. Inward currents were elicited by clamp steps to +10 mV and were completely suppressed by 100 mM propiverine (Fig. 6a,b). $I_{Ca,L}$ amplitudes were not significantly different in juvenile and adult pigs (Fig. 6c). Propiverine reduced $I_{Ca,L}$ with similar potency in both groups, resulting in the following pIC_{50} values [*M*]: 4.57±0.34 (n=7/3) in juvenile versus 4.76±0.25 (n=7/3) in adult cells (Fig. 6d).

Discussion

The aim of the present study was to compare muscarinic receptor-mediated detrusor contraction in juvenile and adult porcine urinary bladder. We found small but consistent differences between juvenile and adult detrusor tissue across the different experimental techniques, which reached statistical significance only in some cases. In particular, we detected that (1) mRNA expression of muscarinic M_2 and M_3 subtypes was similar; (2) albeit somewhat lower in juvenile tissue, the number of muscarinic receptor-binding sites and binding affinity for [^{3}H]QNB were not statistically different; (3) potency of the muscarinic receptor agonist carbachol was slightly but significantly lower in juvenile than adult bladders; and (4) L-type Ca^{2+} currents were nonsignificantly smaller in cells from juvenile than from adult animals. Irrespective of their variable statistical significance, the magnitude of the observed differences typically was so small that it is unlikely to be of clinical relevance when extrapolated to humans.

Muscarinic receptor-mediated contractile function in detrusor muscle has been studied in fetal, newborn, and adult tissue from rat, rabbit, sheep, and bovine [16, 17, 18,

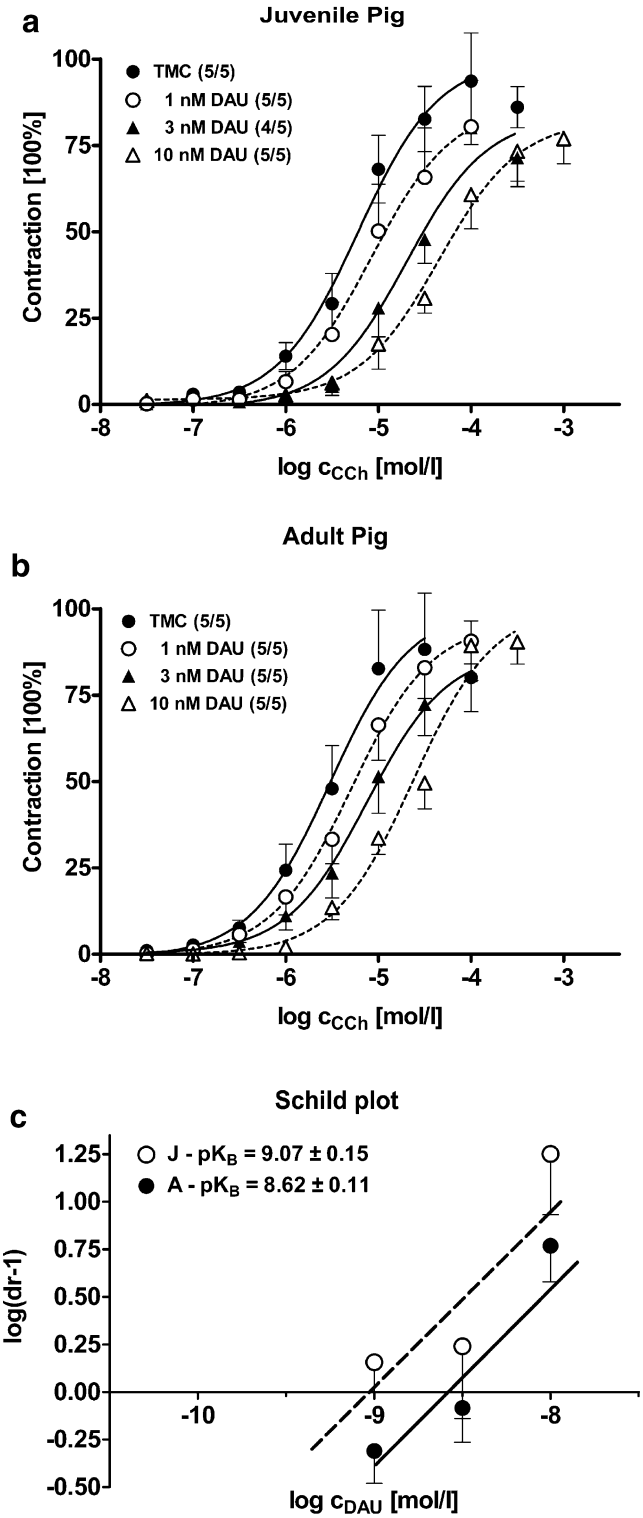

Fig. 3 Cumulative concentration–response curves (*CRC*) for carbachol (*CCh*) in the presence and absence of different concentrations of the M_3 receptor antagonist DAU 5884 (*DAU*) in juvenile (**a**) and adult pigs (**b**). Responses are expressed as percentage of the maximum response during the first CRC for carbachol, and data are presented as mean + SEM from *n* investigated strips from *x* animals. TMC indicates time-matched control experiments without any test drug added. **c** Schild plot for the determination of the apparent affinity for DAU 5884 in juvenile and adult porcine detrusor; slope defined as 1

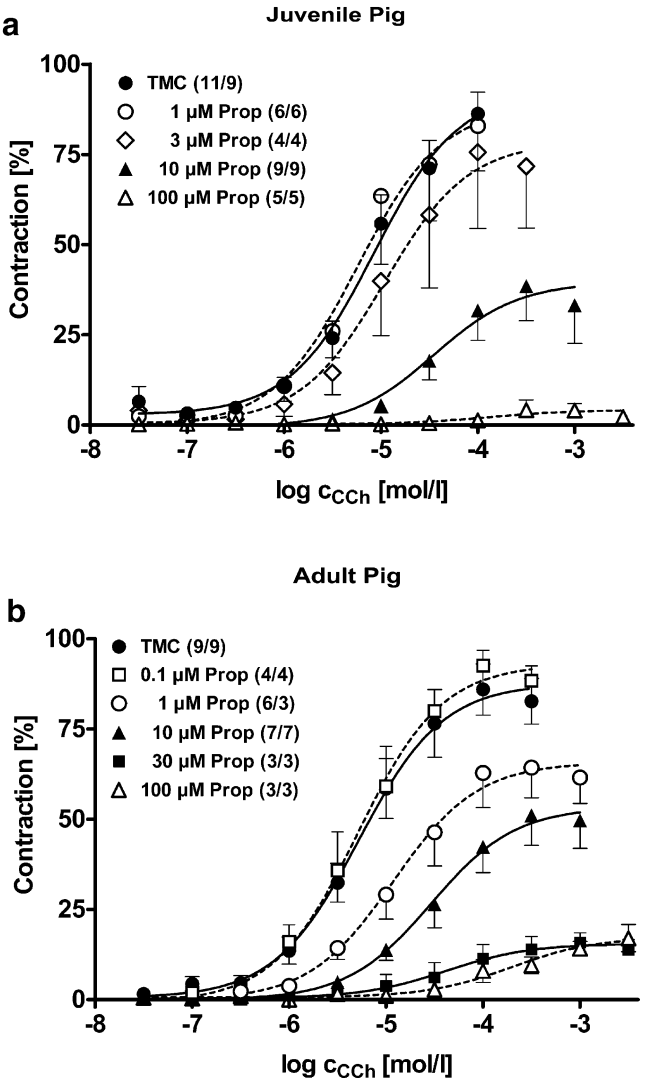

Fig. 4 Cumulative concentration–response curves (*CRC*) for carbachol (*CCh*) in the presence and absence of different concentrations of propiverine in juvenile (**a**) and adult animals (**b**). Data are presented as mean + SEM from *n* investigated strips from *x* animals. Some data at 0.1 µM (4/4), 1 µM (6/3), and 10 µM (4/3) were taken from [39]. Layout like in Fig. 3a, b

32]. These studies revealed a high muscarinic receptor density at birth; no significant changes in the cholinergic innervation over the first 6 weeks of postnatal development in the rabbit [17] but a decrease in potency and efficacy of carbachol and ATP during the first month of newborn rats [32]. So far, only one study has evaluated that muscarinic receptor subtypes are expressed to a significantly greater extent in fetal bladders [5]. In this study, we have studied muscarinic M_2 and M_3 receptor expression and function in juvenile and adult porcine detrusor to compare detrusor contractile function in premature and mature organisms. In fact, as predicted by earlier studies in different species, we did not observe significant differences in the individual receptor subtype expression. However, binding of [^{3}H] QNB tended to be lower in juvenile bladders. This finding

Table 1 Effect of propiverine on cumulative concentration–response curves (CRC) for carbachol in juvenile and adult porcine detrusor

	n	ΔpEC_{50}	Eff_{max} (%)
Juvenile pig			
Time-matched controls	11/9	0.38±0.11	92±7
Propiverine			
1 μM	6/6	0.49±0.16	88±10
3 μM	4/4	0.54±0.44	81±16
10 μM	9/9	1.10±0.17*	42±9***
100 μM	5/5	2.45±0.11***	5±3***
Adult pig			
Time-matched controls	9/9	0.37±0.26	92±6
Propiverine			
0.1 μM	4/4	0.42±0.19	93±4
1 μM	6/3	0.73±0.11*	67±8*
10 μM	7/7	1.31±0.24**	50±9**
30 μM	3/3	1.63±0.23**	16±3***
100 μM	3/3	2.37±0.43***	18±3***

Some data at 0.1 μM (4/4), 1 μM (6/3), and 10 μM (4/3) were taken from [39].

ΔpEC_{50} [M] Difference of the pEC_{50} values between the first and second CRC for carbachol, *Eff_{max}* maximum contraction during the second CRC expressed in percent of the maximum effects during the first CRC (=100%), means ± SEM; *n* number of detrusor strips from *x* animals

*$p<0.05$

**$p<0.01$

***$p<0.001$ (compared to the relevant TMC value)

is in line with the lower potency of the M receptor agonist carbachol in juvenile versus adult tissue. This is in contrast to findings in rats, where the fetal detrusor tissue is more sensitive for carbachol approaching the properties of adult bladders and looses this hypersensitivity 1 month after birth [32]. Given the lower potency of carbachol in juvenile tissue, the observed Eff_{max} was significantly higher than in adult pigs, confirming earlier results in rats, where carbachol induced almost one-third larger contractions in 1-month-old than in 4-month-old detrusor tissue [32].

Binding of the muscarinic antagonist [^{3}H]QNB was in good agreement with carbachol-induced contraction properties, indicating only a small functional difference between juvenile and adult detrusor tissue. In contrast to larger changes in purinergic receptor expression and function during maturation [24], our data support earlier predictions of only small alterations regarding muscarinic receptor function in the bladder.

In addition to direct agonistic receptor stimulation, we have also studied the influence of Ca^{2+} influx through L-type Ca^{2+} channels, as muscarinic receptor-induced detrusor contraction largely depends on this process [22, 26, 28, 35, 38]. Again, as seen in the other experimental approaches, our data show only a small difference in $I_{Ca,L}$ between juvenile and adult porcine detrusor cells, which also did not reach statistical significance. Tugay et al. describe significantly larger contractions induced by 80 mM KCl in the neonatal rat, whereas 1 month after birth, no significant difference is seen in the detrusor as compared to adult rats [32]. Zderic et al. found that bethanechol-induced detrusor contractions in 1-day- and 1-week-old rabbits were more sensitive to increases in extracellular Ca^{2+} and L-type Ca^{2+} channel blockers than from mature 8-week-old rabbits [41, 42]. The authors concluded that during maturation, ryanodine-induced Ca^{2+} released from the sarcoplasmatic reticulum increases rather than the Ca^{2+} influx via L-type Ca^{2+} channels elevated. However, this statement has to be handled with caution because Ca^{2+} influx and Ca^{2+} release processes may vary in

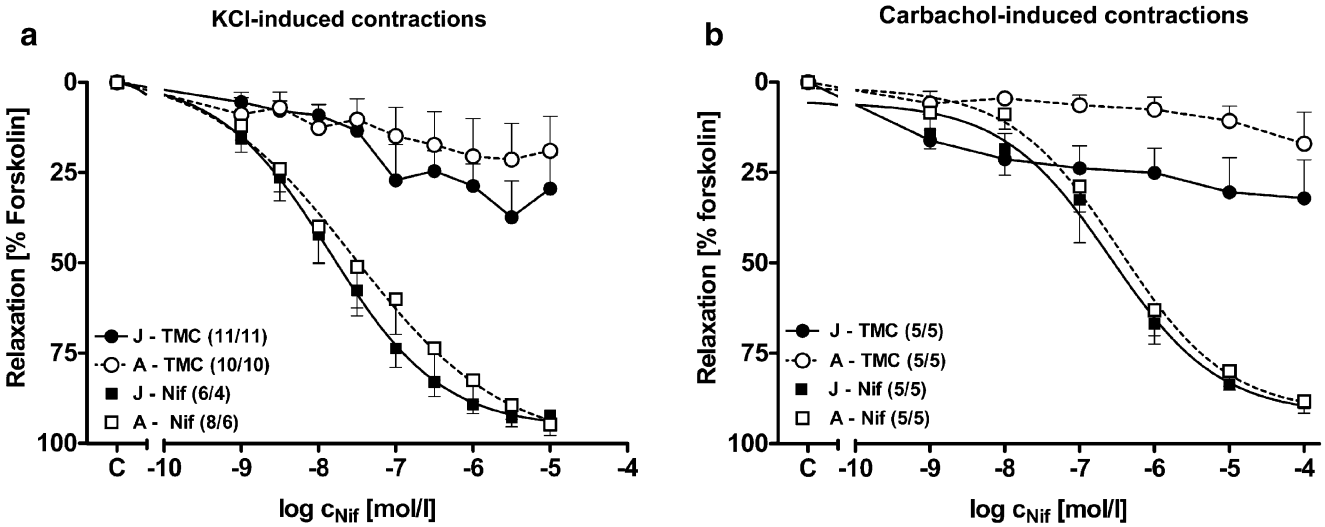

Fig. 5 Effect of cumulatively added concentrations of the L-type Ca^{2+} channel blocker nifedipine [Nif] on detrusor contraction induced by either 40 mM KCl (**a**) or 3 μM carbachol [CCh] (**b**). The concentration–response curves are presented as percent relaxation of 10 μM forskolin [F] (=100%). Data as mean + SEM from *n* detrusor strips from *x* animals. *TMC* indicates time-matched control experiments without any test drug added

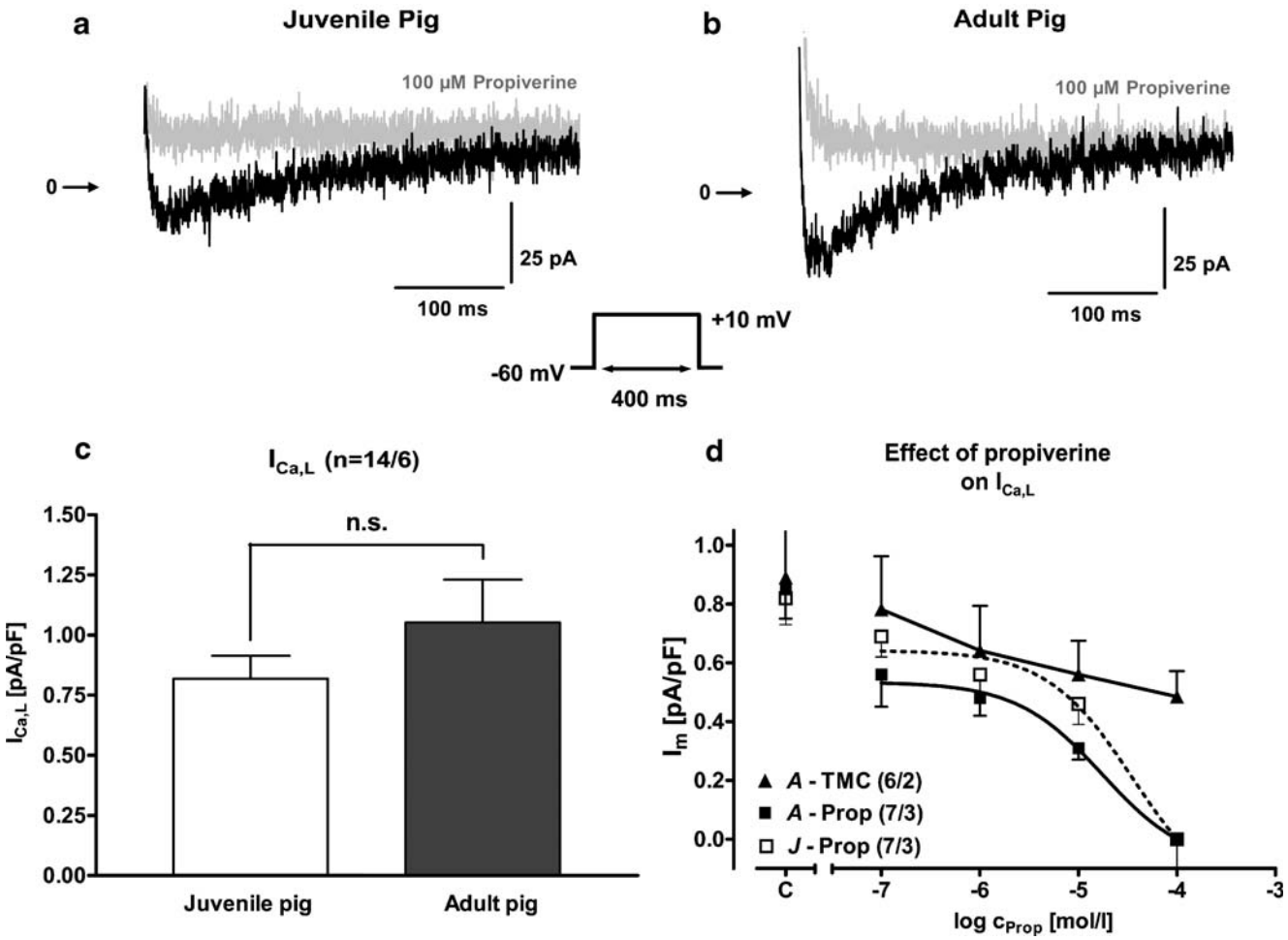

Fig. 6 Current traces for $I_{Ca,L}$ in juvenile (**a**) and adult (**b**) porcine detrusor muscle cells and effect of 100 µM propiverine. Current densities for $I_{Ca,L}$ are presented as pA/pF (**c**). Concentration–response curves for propiverine (*Prop*) on $I_{Ca,L}$ in juvenile (*J*) and adult (*A*) cells are compared to the time course (*TMC*) of the maximum current density in detrusor cells from adult pigs (**d**). Data are mean + SEM from *n* individual cells from *x* animals (**c** and **d**)

different species [26, 28, 38]. In this study, we did not observe any difference in the effect of the L-type Ca^{2+} channel blocker nifedipine, suggesting that Ca^{2+} influx does not change in porcine detrusor during maturation.

Antagonistic properties on muscarinic receptor-induced detrusor contraction were also not significantly changed. The reported affinity (pK_i value) of DAU 5884 at M_3 receptors in submandibular glands is 8.80±0.03 as compared to 7.40±0.05 on cardiac M_2 receptors [8]. Accordingly, 100 nM DAU 5884 yields a fractional M_3 receptor occupancy of 99 versus 26% at M_2 receptors [11]. Under these M_3 receptor subtype selective conditions, DAU 5884 similarly antagonized detrusor contractions in both juvenile and adult tissue, with estimated pK_B values close to the reported pK_i at M_3 receptors. Therefore, M_3 receptors appear to be similarly the predominant subtype contributing to detrusor contraction in juvenile and adult pigs. Our estimated pK_B value of 8.62 for the apparent affinity of DAU 5884 in adult porcine detrusor tissue is in a similar range as the pA_2 value of 8.72 reported for DAU 5884 in guinea pig tracheal tissue known to mediate its contraction exclusively via M_3 receptors [27]. Our data show that expression and function of M_3 receptors seem to be not significantly altered during maturation.

In addition, we have tested whether potency and efficacy of the spasmolytic drug propiverine are different in juvenile and adult detrusor tissue, as it is clinically used to treat children with symptoms of overactive bladder [13, 20, 21, 31]. Antimuscarinic drugs are one therapeutic option to treat children suffering from enuresis and urinary incontinence. Their effectiveness has been demonstrated in several clinical studies [21, 23, 30] despite a particularly high response rate to placebo treatment in this clinical setting. To use current drugs properly or develop more efficacious drugs for treating children with urinary incontinence, further research of the physiological function of the lower urinary tract especially during postnatal development is necessary. In this study, we found that potency and efficacy of propiverine were indeed similar between juvenile and adult tissues. Furthermore, propiverine impaired electrically induced contractions to a

similar extent in juvenile and adult detrusor [36]. Propiverine also reduced Ca^{2+} influx via L-type Ca^{2+} channels by direct binding to the channels [33, 37]. The potency of propiverine to reduce $I_{Ca,L}$ was similar in juvenile and adult cells. The functional characteristics of propiverine as an antimuscarinic compound, which possesses additional properties like direct blockade of L-type Ca^{2+} channels, suggest that neither M_3 receptors functions nor L-type Ca^{2+} channels are altered during postnatal development of urinary bladder.

In conclusion, we did not detect major functional changes for muscarinic receptor-mediated detrusor contraction during maturation. Therefore, antimuscarinic drug actions seem to be similar in juvenile and adult pig detrusor. In analogy to our findings in pig, which has been established as a good model for human urinary bladder, similar responses to antimuscarinic compounds could be expected in human detrusor tissue. However, further investigations are required to show a comparable efficacy of these drugs in younger and adult patients.

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References

1. Abrams P, Andersson KE, Buccafusco JJ, Chapple C, deGroat WC, Fryer AD, Kay G, Laties A, Nathanson NM, Pasricha PJ, Wein AJ (2006) Muscarinic receptors: their distribution and function in body systems, and the implications for treating overactive bladder. Br J Pharmacol 148:565–578
2. Andersson KE, Arner A (2004) Urinary bladder contraction and relaxation: physiology and pathophysiology. Physiol Rev 84:935–986
3. Andersson KE, Yoshida M (2003) Antimuscarinics and the overactive detrusor—which is the main mechanism of action? Eur Urol 43:1–5
4. Arunlakshana O, Schild, (1959) Br J Pharmacol 14:48–58
5. Brandes SB, Ruggieri MR (1995) Adv Exp Med Biol 385:241–249
6. Chess-Williams R, Chapple CR, Yamanishi T, Yasuda K, Sellers DJ (2001) The minor population of M3-receptors mediate contraction of human detrusor muscle in vitro. J Auton Pharmacol 21:243–248
7. Christ GJ, Hodge S (2006) Molecular mechanisms of detrusor and corporal myocyte contraction: identifying targets for pharmacotherapy of bladder and erectile dysfunction. Br J Pharmacol 147: S41–S55
8. Doods HN, Willim KD, Boddeke HWGM, Entzeroth M (1993) Characterization of muscarinic receptors in guinea-pig uterus. Eur J Pharmacol 250:223–230
9. Fetscher C, Fleichmann M, Schmidt M, Krege S, Michel MC (2002) M_3 muscarinic receptors mediate contraction of human urinary bladder. Br J Pharmacol 136:641–644
10. Fry CH, Skennerton D, Wood D, Wu C (2002) The cellular basis of contraction in human detrusor smooth muscle from patients with stable and unstable bladders. Urology 59:3–12
11. Gosens R, Nelemans SA, Groote Bromhaar MM, McKay S, Zaagsma J, Meurs H (2003) Muscarinic M_3-receptors mediate cholinergic synergism of mitogenesis in airway smooth muscle. Am J Respir Cell Mol Biol 28:257–262
12. Graf EM, Bock M, Heubach JF, Zahanich I, Boxberger S, Richter W, Schultz JH, Ravens U (2005) Tissue distribution of a human $Ca_v1.2$ α_1 subunit splice variant with 75 bp insertion. Cell Calcium 38:11–21
13. Grigoleit U, Murtz G, Laschke S, Schuldt M, Goepel M, Kramer G, Stohrer M (2006) Efficacy, tolerability and safety of propiverine hydrochloride in children and adolescents with congenital or traumatic neurogenic detrusor overactivity—a retrospective study. Eur Urol 49:1114–1120
14. Hegde SS (2006) Muscarinic receptors in the bladder: from basic research to therapeutics. Br J Pharmacol 147:S80–S87
15. Lazzeri M (2006) More urological clinical trials in children are needed. Eur Urol 50:662–663
16. Lee JG, Coplen D, Macarak E, Wein AJ, Levin RM (1994) Comparative studies on the ontogeny and autonomic responses of the fetal calf bladder at different stages of development: involvement of nitric oxide on field stimulated relaxation. J Urol 151:1096–1101
17. Levin RM, Malkowicz SB, Jacobowitz D, Wein AJ (1981) The ontogeny of the autonomic innervation and contractile response of the rabbit urinary bladder. J Pharmacol Exp Ther 219:250–257
18. Levin RM, Macarak E, Howard P, Horan P, Kogan BA (2001) The response of fetal sheep bladder tissue to partial outlet obstruction. J Urol 166:1156–1160
19. Longhurst P (2004) Developmental aspects of bladder function. Scand J Urol Nephrol Suppl 215:11–19
20. Madersbacher H, Mürtz G (2001) Efficacy, tolerability and safety profile of propiverine in the treatment of the overactive bladder (non-neurogenic and neurogenic). World J Urol 19:324–335
21. Marschall-Kehrel D, Persson-de Geeter C, Stehr M, Simson G, Matschke U, Veticka J, Ionescu S, Sillén U, Martincok D, Radmayr C, Nijman J, Schmidt C (2007) Study design As the main predictor for good outcome in a randomised placebo-controlled multicentre trial in children suffering from overactive bladder and urinary incontinence. Neurourol Urodyn 26:657–658
22. Masters JG, Neal DE, Gillespie JI (1999) The contribution of intracellular Ca^{2+} release to the contraction in human bladder smooth muscle. Br J Pharmacol 127:996–1002
23. Nijman RJ (2004) Role of antimuscarinics in the treatment of nonneurogenic daytime urinary incontinence in children. Urology 63:45–50
24. O'Reilly BA, Kosaka AH, Chang TK, Ford APDW, Popert R, Rymer JM, McMahon SB (2001) A quantitative analysis of purinoceptor expression in human fetal and adult bladders. J Urol 165:1730–1734
25. Ouslander JG (2004) Management of overactive bladder. N Engl J Med 350:786–799
26. Rivera L, Brading AF (2006) The role of Ca^{2+} influx and intracellular Ca^{2+} release in the muscarinic-mediated contraction of mammalian urinary bladder smooth muscle. BJU Int 98:868–875
27. Roffel AF, Elzinga CR, Zaagsma J (1993) Cholinergic contraction of the guinea pig lung strip is mediated by muscarinic M_2-like receptors. Eur J Pharmacol 250:267–279
28. Schneider T, Fetscher C, Krege S, Michel MC (2004) J Pharmacol Exp Ther 309:1148–1153
29. Schneider T, Hein P, Michel-Reher MB, Michel MC (2005) Effects of ageing on muscarinic receptor subtypes and function in rat urinary bladder. Naunyn Schmiedeberg's Arch Pharmacol 372:71–78
30. Schulte-Baukloh H, Murtz G, Henne T, Michael T, Miller K, Knispel HH (2006) Urodynamic effects of propiverine hydrochloride in children with neurogenic detrusor overactivity: a prospective analysis. BJU Int 97:355–358
31. Schultz-Lampel D (2006) The overactive bladder during childhood. When and how should it be treated? Urologe A 45:841–846

32. Tugay M, Yildiz F, Utkan T, Gacar N, Ulak G, Eden F (2003) Age-related smooth muscle reactivity changes in the rat bladder: an in vitro study. Pharmacol Res 48:329–334
33. Tokuno H, Chowdhury JU, Tomita T (1993) Inhibitory effects of propiverine on rat and guinea-pig urinary bladder muscle. Naunyn Schmiedeberg's Arch Pharmacol 348:659–662
34. Uchida S, Kurosawa S, Fujino Oki T, Kato Y, Nanri M, Yoshida K, Yamada S (2007) Binding activities by propiverine and its N-oxide metabolites of L-type calcium channel antagonist receptors in the rat bladder and brain. Life Sci 80:2454–2460
35. Wegener JW, Schulla V, Lee TS, Koller A, Feil S, Feil R, Kleppisch T, Klugbauer N, Moosmang S, Welling A, Hofmann F (2004) FASEB J 18:1159–1161
36. Wuest M, Braeter M, Schoeberl C, Ravens U (2005) Juvenile pig detrusor: effects of propiverine and three of its metabolites. Eur J Pharmacol 524:145–148
37. Wuest M, Hecht J, Christ T, Braeter M, Schoeberl C, Hakenberg OW, Wirth MP, Ravens U (2005) Pharmacodynamics of propiverine and three of its main metabolites on detrusor contraction. Br J Pharmacol 145:608–619
38. Wuest M, Hiller N, Braeter M, Hakenberg OW, Wirth MP, Ravens U (2007) Contribution of Ca^{2+}. influx to carbachol-induced detrusor contraction is different in human urinary bladder compared to pig and mouse. Eur J Pharmacol 565:180–189
39. Wust M, Averbeck B, Reif S, Brater M, Ravens U (2002) Different. Eur J Pharmacol 454:59–69
40. Yamanishi T, Chapple CR, Yasuda K, Chess-Williams R (2000) The role of M_2 muscarinic receptors in mediating contraction of the pig urinary bladder in vitro. Br J Pharmacol 131:1482–1488
41. Zderic SA, Hypolite J, Duckett JW, Snyder HM, Wein AJ, Levin RM (1991) Development aspects of bladder contractile function: sensitivity to extracelluar calcium. Pharmacology 43:61–68
42. Zderic SA, Sillen U, Liu GH, Liu GH, Snyder HM 3rd, Duckett JW, Gong C, Levin RM (1994) Developmental aspects of excitation contraction coupling of rabbit bladder smooth muscle. J Urol 152:679–681