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MUSCARINIC RECEPTOR-MEDIATED CONTRACTION IN JUVENILE AND ADULT PORCINE DETRUSOR

Hypothesis / aims of study

Antimuscarinic drugs are one option to treat children suffering from enuresis and urinary incontinence, and have proven beneficial in several clinical studies. Since experimental investigations on the muscarinic receptor system of the urinary bladder have been largely limited to adult animals, we have compared the muscarinic receptor-mediated contraction in juvenile and mature porcine detrusor.

Study design, materials and methods

Urinary bladders of juvenile (8-12 weeks; 12 to 35 kg body weight) and mature farm pigs (>40 weeks; >100 kg) were used. The mRNA expression of the two muscarinic receptor suptypes M_2 and M_3 was determined with realtime-PCR using an internal standard. Receptor protein expression was assessed by radioligand binding experiments with [^{3}H]quinuclidinyl benzylate (QNB). Muscarinic receptor-mediated detrusor contraction was measured in response to the agonist carbachol. Cumulative concentration-response curves (CRC) for carbachol were generated in the presence and absence of different concentrations of the M_3-selective antagonist DAU 5884 (8-Methyl-8-azabicyclo-3-*endo*[3.2.1]oct-3-yl-1,4-dihydro-2-oxo-3(2*H*)-quinazoline-carboxylic acid ester hydrochloride) or the non-selective muscarinic receptor antagonist and spasmolytic drug propiverine and compared to CRC for carbachol without any substance added (time controls).

Results

The expression of mRNA was similar in adult and juvenile porcine detrusor for M_2 (16 ± 7 vs.18 ± 5 fg/ng, n = 8-10) and M_3 receptors (32 ± 12 vs. 39 ± 16 fg/ng, n = 10-12). The number of [^{3}H]QNB binding sites (B_{max} 48.9 ± 8.7 vs. 32.3 ± 3.3 fmol/mg) and their affinity for the radioligand (K_D 7.6 ± 2.1 vs. 6.8 ± 2.9 pmol/l, n = 7 each) were not significantly different in adult and juvenile pigs. In contrast, potency of the muscarinic receptor agonist carbachol was sligthly different in adult and juvenile pigs: **-**log EC_{50} [M] 5.80 ± 0.06 (n = 52/14) versus 5.52 ± 0.07 (n = 54/14; $p < 0.01$).

Table 1 Δ–*log* EC_{50} [M], difference of the negative logarithm of carbachol concentration for half maximum effect between a 1st and 2nd CRC for carbachol; Means ± S.E.M., *n* = number of detrusor strips from y animals; * $p < 0.001$, # $p < 0.05$, + $p < 0.001$ (compared to the relevant *time control* value).

	n	*Δ-log* EC_{50}
adult pig		
time controls	5/5	0.48 ± 0.03 *
DAU 5884		
1 nM	5/5	0.49 ± 0.08
3 nM	5/5	0.70 ± 0.12
10 nM	5/5	1.34 ± 0.03 *
juvenile pig		
time controls	5/5	0.44 ± 0.15 # +
DAU 5884		
1 nM	5/5	0.51 ± 0.20
3 nM	4/4	0.98 ± 0.20 #
10 nM	5/5	1.29 ± 0.31 +

The M_3 antagonist DAU 5884 (1-10 nM) concentration-dependently shifted the CRC for carbachol to higher concentrations (Table 1). The calculated pK_B values for DAU 5884 as a measure for affinity of the antagonist were 9.16 ± 0.21 (adult) and 9.28 ± 0.17 (juvenile).
The spasmolytic drug propiverine (0.1-100 µM) also shifted the CRC for carbachol to higher concentrations and, in addition, it reduced the maximum contractions (Eff_{max}). Potency and efficacy of propiverine were similar in both adult and juvenile tissue (Table 2).

Table 2 *Δ–log EC_{50}* [M], difference of the negative logarithm of carbachol concentration for half maximum effect between a 1st and 2nd CRC for carbachol and *Eff_{max}*, maximum contraction during the 2nd CRC expressed in percent of the maximum effects during the 1st CRC (=100%); Means ± S.E.M., *n* = number of detrusor strips from y animals, # p < 0.05; * p < 0.05 ** p < 0.01 *** p < 0.001 (compared to the relevant *time control* value).

	n	*Δ-log EC_{50}*	*Eff_{max} [%]*
adult pig			
time 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***
juvenile pig			
time 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***

Interpretation of results
The mRNA expression of M_2 and M_3 receptors and the binding properties for [^{3}H]QNB were not significantly different between juvenile and adult porcine detrusor tissue, although number of muscarinic receptor binding sites and binding affinity for [^{3}H]QNB tended to be lower in the juvenile tissue. This latter observation on the protein level would be in line with the functional measurements for the potency of the muscarinic receptor agonist carbachol, which was significantly less potent in the juvenile detrusor. Despite these differences regarding the agonistic stimulation of detrusor contraction, the affinities for the M_3 receptor antagonist DAU 5884 were similar indicating no difference for the relaxation in the presence of an muscarinic receptor antagonist. While potency and efficacy for the spasmolytic drug propiverine were comparable in juvenile and adult tissue, similar antagonistic properties on muscarinic receptors are predicted in both type of detrusor tissue.
Concluding message
The expression and function of M_2 and M_3 receptors seem to be similar in the detrusor of juvenile and mature pigs, although a tendency for lower receptor binding and lower potency for carbachol was found in juvenile detrusor. Our data with muscarinic receptor antagonists suggest that the pharmacodynamic properties of spasmolytic drugs such as propiverine should be similar in young and adult patients with urinary bladder dysfunction.

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