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1. UROsphere

FUNCTIONAL IMPORTANCE OF CHOLINERGIC AND PURINERGIC NEUROTRANSMISSIONS FOR MICTURITION REFLEX IN ANESTHETIZED MICE.

Hypothesis / aims of study

In humans, the micturition reflex is mainly mediated by the cholinergic pathway. However, in pathological conditions, such as interstitial cystitis or bladder instability (1, 2), an implication of a purinergic component has been reported. A previous *in vitro* study in mice isolated urinary bladder showed that neurogenic-mediated contractions are mainly purinergic and the residual contraction is mediated by muscarinic receptors (3). However, there is no information about implication of acetylcholine and ATP on the micturition reflex in mice. Therefore, the aim of the present study was to evaluate the role of cholinergic and purinergic neurotransmission on the micturition reflex in anesthetized mice.

Study design, materials and methods

Discontinuous cystometries were performed in female C57BL/6J mice. A catheter connected to a pressure transducer was implanted into the bladder. Bladder was perfused (NaCl 0.9%, 0.6 mL/h) until inducing a micturition cycle, then the bladder was manually emptied to measure residual volume. A series of three control micturition cycles (considered as basal values) were obtained. Then, atropine (ATR, 0.3 mg/kg), PPADS (a purinergic antagonist, 30 mg/kg), ATR + PPADS or vehicle (NaCl 0.9%) were administered (i.v. route). The effects of drugs were observed on two consecutive micturition cycles, 5 min after drugs administration. Amplitude of Micturition (AM, mmHg), Bladder Capacity (BC, mL), Residual Volume (RV, mg), Frequency (FSA, n/min) and Amplitude (ASA, mmHg) of Spontaneous Activity were analyzed. The results were expressed as mean ± s.e.m. as percentages of variation from basal values. The differences between cystometric parameters before and after drugs administration were statistically compared.

Results

In all groups, reproducible control micturition cycles were observed. Cystometric basal values between groups were not statistically different ($p>0.05$, one-way ANOVA). Vehicle was without any significant effect on all cystometric parameters. ATR (n=6) and PPADS (n=6) significantly decreased AM and increased RV (see Table). In addition, ATR significantly increased BC. Neither FSA nor ASA were modified by ATR and PPADS (data not shown). ATR + PPADS induced dribbling incontinence in all animals (n=6).

Interpretation of results

In anesthetized mice, cholinergic and purinergic neurotransmissions were equally implicated on the efferent pathway as reflected by the same level of AM inhibition by ATR or PPADS. The effect on AM of both treatments probably explains the RV increase. Moreover, cholinergic neurotransmission was implicated on the afferent pathway since ATR increased BC suggesting a role for muscarinic receptors located on bladder urothelium.

Concluding message

The dribbling incontinence observed after simultaneous administration of ATR and PPADS support the view that the two main neurotransmitters implicated on micturition reflex in mice are acetylcholine and ATP. This experimental model could be useful to evaluate the effects of new muscarinic and purinergic receptor antagonists in order to discover new treatments for overactive bladder in humans.

References

1. J Urol (1993) 150; 2007-2012
2. J Urol (2002) 167; 157-164
3. Br J Pharmacol (2000) 131;1489-1495

Table

	Mean AM ± SEM	Mean BC ± SEM	Mean RV ± SEM
	(mmHg)	(mL)	(mg)
NaCl 0.9% (i.v., n=7):			
Before	28 ± 2	0.090 ± 0.012	74 ± 10
After	26 ± 2	0.081 ± 0.009	66 ± 9
%	-5.1 ± 5.1	-5.2 ± 9.0	-6.5 ± 8.5
Atropine (0.3 mg/kg, i.v., n=6):			
Before	26 ± 3	0.071 ± 0.007	58 ± 11
After	18 ± 2*	0.092 ± 0.007**	87 ± 6**
%	-30.0 ± 6.8	+31.0 ± 6.7	+69.0 ± 22.0
PPADS (30 mg/kg, i.v., n=6):			
Before	21 ± 3	0.077 ± 0.012	61 ± 11
After	14 ± 3*	0.089 ± 0.009	82 ± 10***
%	-32.6 ± 10.2	+21.0 ± 9.0	+40.7 ± 8.9
Atropine + PPADS (i.v., n=6):			
Before	24 ± 3	0.077 ± 0.004	62 ± 7
After (1)			

Paired Student *t*-test. *p<0.05; **p<0.01; ***p<0.001 *vs* basal values.
(1) Absence of the micturition reflex.

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