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

Stefano Palea, Aurore Deba, Philippe Lluel
UROsphere - Faculty of Pharmaceutical Sciences, Toulouse, France

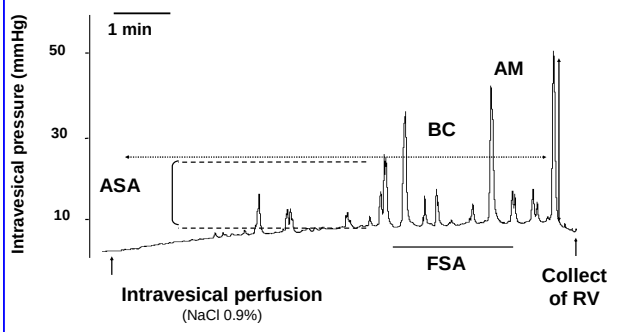
INTRODUCTION & OBJECTIVE

- In Humans, the micturition reflex is mainly mediated by the cholinergic pathway.
- In pathological conditions, such as interstitial cystitis (1) or bladder instability (2) or during aging (3) an implication of a purinergic component has been reported in human isolated bladder smooth muscle.
- In mice isolated urinary bladder, the contractions are mainly mediated by acetylcholine (Ach) and ATP (4). However, relative implication of cholinergic and purinergic component has not definitively demonstrated *in vivo*.
- The aim of this study was to evaluate the functional importance of Ach and ATP in micturition reflex in female anesthetized mice.

MATERIALS & METHODS

- Discontinuous cystometries were performed in female C57Bl6/J mice anesthetized with urethane (1.8 g/kg, i.p.). A catheter connected to a pressure transducer was implanted into the bladder to record intravesical pressure. A jugular vein was catheterized for compound administration.
- Bladder was perfused (NaCl 0.9%, 0.6 mL/h) until a micturition cycle occurs. Then the bladder was manually emptied, and the residual volume measured. After 3 micturition cycles (considered as basal values), atropine (ATR, 0.3 mg/kg), PPADS (a purinergic antagonist, 30 mg/kg), the association of ATR + PPADS or vehicle (NaCl 0.9%) were administered intravenously. The effects of compounds were observed on two consecutive micturition cycles, 5 min after administration. Cystometric parameters analyzed are presented on Figure 1.
- The effects of compounds on micturition cycles were expressed as mean ± sem of percentages of variation from basal values.
- Statistical comparison was done by paired Student *t* test. A p< 0.05 was accepted for significance.

Figure 1: Typical recording of a micturition cycle and cystometric parameters analyzed in female anesthetized mice.



AM: Amplitude of Micturition
BC: Bladder Capacity
RV: Residual Volume
ASA: Amplitude Spontaneous Activity
FSA : Frequency Spontaneous Activity

BASAL VALUES

In all groups, micturition cycles were reproducible and cystometric basal values between groups were not statistically different (p>0.05, data not shown).

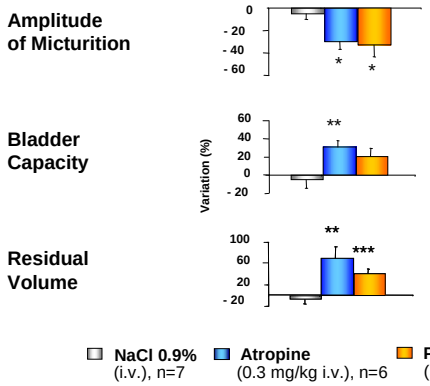
EFFECTS OF VEHICLE ON CYSTOMETRIC PARAMETERS

Vehicle (n=7) was without any significant effect on all cystometric parameters.

EFFECTS OF ATROPINE, PPADS AND THEIR ASSOCIATION ON CYSTOMETRIC PARAMETERS

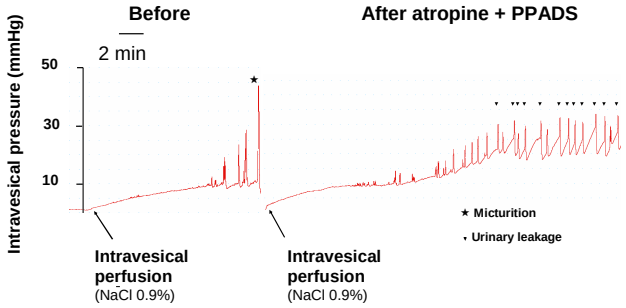
ATR (n=6) and PPADS (n=6) significantly decreased AM and increased RV. In addition, ATR increased BC. Although BC was increased after PPADS administration, the result did not reach significance (Figure 2).
Neither FSA nor ASA were modified by ATR and PPADS (data not shown). ATR + PPADS (n=6) induced dribbling incontinence in all animals (Figure 3).

*Figure 2: Effects of vehicle, atropine and PPADS on cystometric parameters (*p<0.05, **p<0.01, ***p<0.001 vs basal values).*



Basal values (n=19)
AM : 25.0 ± 0.2 mmHg
BC : 0.079 ± 0.002 mL
RV : 0.064 ± 0.001 g

Figure 3: Effects of atropine (0.3 mg/kg i.v.) + PPADS (30 mg/kg i.v.) on micturition cycle in anesthetized mice.



DISCUSSION

- 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 and PPADS. This effect on AM could explains the increase RV observed.
- Cholinergic neurotransmission was implicated on the afferent pathway since ATR increase BC supporting a role for muscarinic receptors located on bladder urothelium as recently reported (5).

CONCLUSION

- Dribbling incontinence after ATR+PPADS showed the physiological importance of acetylcholine and ATP in micturition reflex in mice.
- The mice may provide a model for the understanding of bladder function and could be useful to investigate and develop pharmacological treatments for urinary incontinence in Humans.

REFERENCES

1. Palea S *et al.* J Urol. **150**: 2007-12, 1993.
2. O'Reilly BA *et al.* J Urol. **167**: 157-64, 2002.
3. Yoshida M *et al.* Exp Gerontol. **36**: 99-109, 2001.
4. Vial C *et al.* Br J Pharmacol. **131**: 1489-1495, 2000.
5. Mukerji *et al.* J Urol. **176**: 367-373, 2006.

UROsphere