

## IN VIVO AND IN VITRO FUNCTIONAL CHANGES OF THE URINARY BLADDER IN MICE LACKING MUSCARINIC M2 OR M3 RECEPTORS

### Aims of Study

Detrusor muscle function has been studied previously in vitro in both M<sub>3</sub> and M<sub>2</sub> muscarinic receptor knockout mice, and important gender differences have been suggested [1,2]. However, the urodynamic consequences of lack of M<sub>3</sub> or M<sub>2</sub> receptors have not been investigated. Studies in mice lacking muscarinic receptor subtypes may offer a possibility to obtain information on which muscarinic receptor subtype is the most important for bladder emptying in vivo, and to what extent non-muscarinic mechanisms can compensate for lack of muscarinic receptor functions. In the present study, bladder function was studied by cystometry and detrusor smooth muscle responses to stimulation of muscarinic and purinergic receptors and to electrical field stimulation (EFS) were investigated in mutant mice lacking M<sub>2</sub> or M<sub>3</sub> receptors.

### Methods

Male and female mice lacking M<sub>3</sub> (M<sub>3</sub> KO) or M<sub>2</sub> (M<sub>2</sub> KO) receptors and matching wild type controls (WT), 18-23 weeks of age, were used. For cystometric studies, a polyethylene catheter (PE-50) was implanted into the bladder, and a separate polyethylene catheter was implanted in the subcutaneous layer of the back for drug administration. Cystometric investigations were performed without any anesthesia 3 days after the operation. Room-temperature saline was instilled into the bladder at a rate of 2.5 ml.h<sup>-1</sup>. Intravesical pressure and micturition volumes were recorded continuously before and after subcutaneous administration of atropine sulphate (1mg.kg<sup>-1</sup>). In vitro responses of isolated detrusor muscle strips with intact urothelium to carbachol, ??-methylene ATP (??-Me-ATP) and EFS were also investigated, and are shown as percentages of the maximal contractile response to 118 mM KCl. A Student's paired two-tailed t-test was used for comparisons between before and after drug administration. Student's unpaired two-tailed t-test and one-way factorial ANOVA followed by Scheffe's F-test were used for comparisons between groups. A probability level of <5% was accepted as significant.

### Results

*In vivo studies.* In males, there was no significant difference in any of the cystometric parameters between WT and M<sub>2</sub> KO mice, but M<sub>3</sub> KO mice had significantly longer voiding intervals and larger micturition volumes and bladder capacity than WT and M<sub>2</sub> KO mice. In females, both M<sub>3</sub> KO mice and M<sub>2</sub> KO mice had significantly longer voiding intervals and larger micturition volumes than WT mice. There was no difference in post-void residual volumes among these 3 groups. In the WT and M<sub>2</sub> KO mice of both genders, atropine significantly decreased threshold and micturition pressures, voiding intervals and micturition volumes, and significantly increased residual volumes and bladder capacity. On the other hand, in the M<sub>3</sub> KO mice of both genders, atropine had no effect on any of the parameters.

*In vitro studies.* Carbachol concentration-dependently contracted the bladder strips of WT and M<sub>2</sub> KO mice of both genders: the maximal contractions, obtained at 10 ? M, were 127±4% and 114±4% in male and female WT mice bladders and 109±4% and 106±5% in male and female M<sub>2</sub> KO mice bladders, respectively. In contrast, those values of male and female M<sub>3</sub> KO mice bladders were only 8.9±2.2% and 3.9±0.3%, respectively, which were significantly lower than those obtained from WT and M<sub>2</sub> KO mice. ??-Me-ATP (0.1– 100 ? M) elicited concentration-dependent contractions of the bladder strips from all types of mice. In preparations from male, but not female, M<sub>3</sub> KO mice, the ??-Me-ATP-induced bladder contractions were more pronounced than those from WT and M<sub>2</sub> KO mice. EFS produced frequency-dependent contractile responses in all the three groups. The maximal contractile responses to EFS in male and female WT mice bladders were 131±11% and 125±8%, respectively, which were significantly higher than those (68±8%; p<0.01, males; 75±5%; p < 0.01, females) for M<sub>3</sub> KO mice bladders. The corresponding values for male and female M<sub>2</sub> KO mice bladders were

101±15% and 101±8%, respectively, which were not significantly different from those of WT mice bladders. After 1  $\mu$ M atropine treatment, the EFS-induced contractions were inhibited by 50% and 33% in male and female WT mice bladders, and by 47% and 30% in male and female  $M_2$  KO mice bladders, respectively, but were not affected in  $M_3$  KO mice bladders.  $\alpha$ -Me-ATP (30  $\mu$ M) markedly diminished the atropine-resistant component, and eventually the remaining component in the presence of both atropine (1  $\mu$ M) and  $\alpha$ -Me-ATP (30  $\mu$ M) was not further suppressed by addition of TTX (1  $\mu$ M) in all groups.

### **Conclusions**

The present results confirm that  $M_3$  receptors are the main muscarinic receptor subtype responsible for bladder contraction during voiding, and that the role of  $M_2$  receptors is minor. They also show that muscarinic receptors, at least in mice, are not necessary for bladder emptying, suggesting that the non-cholinergic part of bladder contraction can compensate for a defect in the muscarinic mechanisms.

### **References**

1. Proc. Natl. Acad. Sci. USA, 2000, 97, 9579-9584.
2. J Neurosci 2002, 22:10627-10632.